Research Article

Effect of the Application of 1-Methylcyclopropene and Wax Emulsions on Proximate Analysis and Some Antioxidants of Soursop (Annona muricata L.)

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The effect of the application of 1-methylcyclopropene (1-MCP) and wax emulsions, alone or combined, on composition analysis, vitamin C, polyphenols, and antioxidant capacity of soursop was evaluated. Fruits were stored as follows: at 25°C (control), and at 16°C: fruits sprayed with candelilla or flava emulsions, fruits treated with 1500 nL/L of 1-MCP (20°C, 12 h), and fruits treated with 1-MCP and then sprayed with emulsions. Fruits were allowed to ripen and the edible part was used for analysis. Only fruits stored at 16°C without 1-MCP showed visible symptoms of chilling injury. Fruits treated with 1-MCP combined with flava emulsion maintained in greater extent their vitamin C content, dietary fiber, total phenolics content, and antioxidant activity. The combination of 1-MCP and emulsions can be utilized in postharvest handling of soursop because this combination can preserve its nutritional composition and antioxidant activity.

1. Introduction

Soursop (Annona muricata L.) is a tropical, aromatic, sweet, and great tasting fruit. Mexico is the largest producer in the world and the state of Nayarit is the main producer with 13,022 ton per year. Postharvest losses of soursop of up to 60% have been reported [1]; therefore there is a continuous interest to investigate postharvest treatments that may increase shelf life and maintain the nutritional quality of soursop.

Edible coatings applied on fruits create a semipermeable barrier for respiration gases (O2 and CO2) and water vapor and consequently reduce both the respiration rate and the ethylene production rate [2]. 1-MCP is commonly employed as an effective inhibitor of ethylene to prevent the cascade of reactions leading to ripening and senescence [3]. In the past few years, technologies have been reported whereby physiological and physicochemical variables during the ripening process of diverse fruits including soursop were evaluated [4–6]. However, to maintain the physiological and physicochemical quality in fruits, it is important that postharvest technologies preserve nutrients such as vitamins, minerals, fiber, and other bioactive compounds. These components contribute to maintain vital functions of the human body and decrease chronic and degenerative processes, such as cardiovascular diseases and cancer [7]. Therefore, in recent years research on the effects of postharvest treatments on nutritional composition and antioxidants content in fruits has been conducted in order to increase their added value.

Changes on bioactive compounds and antioxidant activity as a result of application of edible coatings have not been studied in soursop. However, studies in other fruits such as papaya and mango are available. Adetuyi et al. [8] reported that the use of edible coatings on papaya preserved antioxidants such as polyphenols, tannins, and ascorbic acid...
during storage. Robles-Sánchez et al. [9] proposed that an alginate-based edible coating maintained color and increased vitamin C content in precut mango cubes. Additionally, there are very few reports on the effect of 1-MCP on nutrients or bioactive compounds. Several studies have demonstrated that 1-MCP reduced the loss of vitamin C in China fruit (Zizyphus jujuba) and pineapples because it partly inhibited the activity of oxidative enzymes [10, 11]. The same effect was observed in tomatoes and apples, in which phenolic compounds were found in greater amounts in fruits treated with 1-MCP, with respect to control fruits, by reduction of polyphenoloxidase activity [12]. In this work we studied the effect of 1-methylcyclopropene and application of emulsions, alone or combined, on proximate analysis and some antioxidant compounds in soursop.

2. Materials and Methods

1-MCP was a kind donation from AgroFresh Inc. (Philadelphia, PA, USA). Candelilla or flava emulsions were donated from Multiceras S. A. de C. V. (Monterrey Nuevo León, Mexico). All solvents, salts, and acids were purchased from Sigma Chemical Co. (St. Louis, MO) or J. T. Baker (Mexico City). All aqueous solutions were prepared with distilled water.

2.1. Plant Material. Soursop fruits were obtained from an orchard located in the village of El Tonino, municipality of Compostela, state of Nayarit, Mexico. Fruits were harvested when their skin was light green; spines were separated and uniform in size. After harvest, fruits were washed and immersed for 5 min in a solution containing 20 mg/L of 2-(4-thiazolyl)-1H-benzimidazole to prevent fungal growth. Fruits were air-dried at 25°C and a relative humidity (RH) of 85–90%.

2.2. Treatments. Lots of 20 fruits were used for each of the treatments as follows.

1. Fruits stored at 25°C (Control).
2. Fruits stored at 16°C without 1-MCP (16°C).
3. Fruits treated with 1500 nL/L of 1-MCP (for 12 h at 20°C) and then stored at 16°C (1-MCP).
4. Fruits treated with candelilla or flavo emulsions diluted with water 10:90 v/v and then stored at 16°C (C10:90 or F10:90).
5. Fruits treated with 1-MCP, immediately applied with emulsions, and then stored at 16°C as mentioned above (1-MCP + C10:90 and 1-MCP + F10:90).

The 1-MCP treatment was applied at 20°C on the same day when fruits were harvested. Exposure to 1-MCP (1500 nL/L diluted in distilled water) was performed in a 225 L sealed chamber fitted with a fan. A 10 mL glass container was used to hold 1-MCP and placed at the bottom of the chamber. The chamber was kept sealed for 12 h and a fan was used to homogenize the release of 1-MCP. Emulsions were diluted with water in a 10:90 v/v ratio and were sprayed, alone or after the 1-MCP application, twice on the surface of fruits, and then air-dried.

All fruits were stored during their ripening stage (6 days for fruits stored 25°C, 9 days for fruits stored at 16°C with and without emulsions, 12 and 14 days for fruits stored at 16°C with 1-MCP alone or combined with emulsions), and the edible part or pulp of 20 fruits per treatment was manually extracted and seeds were removed. The pulp was freeze-dried and stored at −75°C until analyzed. Lyophilized pulp was divided into two lots for treatment and analyses were performed in triplicate.

2.3. Proximate Analysis. Moisture (Method 925.09), protein (Method 920.87), fat (920.39), and ash (Method 923.03) contents were determined following the official AOAC methods [13]. Soluble sugars were quantified by the phenol-sulfuric method [14]. All data, except moisture (g/100 g fresh weight of edible food, FW), were reported as g/100 g dry matter (DM) of the edible portion.

Total dietary fiber (TDF) is the sum of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). It was analyzed by the AOAC enzymatic-gravimetric method (Method 991.42 modified by Mañas and Saura-Calixto [15]. Samples were treated with heat stable α-amylase, protease, and amyloglucosidase to remove protein and starch. Remaining residues were separated by centrifugation (15 min, 25°C, 3000 × g) to separate soluble and insoluble fractions. The supernatants were dialyzed with water to avoid losses of SDF. The dialysates (containing SDF) and residues (nonstarch polysaccharides, NSP) obtained from centrifugation were submitted to acid hydrolysis with sulfuric acid (12 M). To calculate SDF and NSP the amount of glucose obtained by hydrolysis was measured using the method of Englyst & Cummings [16]. Remaining residues were quantified gravimetrically as Klasson lignin (KL). IDF was calculated as the sum of NSP and KL. Data were reported in g glucose/100 g DM.

2.4. Vitamin C. Vitamin C was analyzed using the technique reported by Suntornsuk et al. [17]. Briefly, 10 g of soursop pulp were homogenized with 25 mL of sulfuric acid 2 N, 25 mL of distilled water, and 3 mL of starch solution as indicator. The mixture was titrated with iodine 0.05 N. Data were reported in mg ascorbic acid/100 g FW.

2.5. Total Phenolic Content. Total phenolic content is considered as the sum of extractable polyphenols (EP) and nonextractable polyphenols (NEP). EP was extracted by shaking at room temperature with acidic methanol (HCl 0.8%)-water in ratio 50:50 (v/v) for 1 h and acetone-water (70:30 v/v) for 1 h [18]. After centrifugation (15 min, 25°C, 3000 × g) supernatants were combined and EP was determined with the Folin-Ciocalteu reagent [19]. Data were reported in gallic acid equivalents (GAE)/100 g DM. NEP is divided in condensed tannins (CT) and hydrolysable polyphenols (HP). Both were measured in the freeze-dried pulp that was extracted with methanol/acetone/water above mentioned. CT was determined in the residue with a hydrolysis treatment
The low soluble sugar content in the pulp of fruits stored at 25°C was probably caused by the reduction in starch degradation by a low oxidative capacity of the mitochondria [29] and also produced chilling injury symptoms. This coincided with other studies like that by Vishnu Prasanna et al. [30] who reported that the carbohydrate content of cherimoya decreased when fruits were stored at 10–15°C. With respect to 1-MCP, 1-MCP + ClO:90, and 1-MCP + F10:90 fruits, chilling injury symptoms were not observed and fruits ripened normally, but soluble sugars were statistically smaller (P < 0.05) than in control fruits (25°C) and greater than fruits that were stored at 16°C without 1-MCP. It could be caused by the synergic effect of 1-MCP and emulsions applied [31].

Soluble sugars in fruits is composed mainly of metabolic enzymes; hence, protein concentration increased during ripening of fruits because numerous enzymes are synthetized. The low protein content found in the refrigerated fruit pulp may be attributed to decreased metabolic rate caused by the postharvest treatments and refrigerated storage. The soursop fruit showed a reduction in ethylene production rate [25] and probably had a decreased protein synthesis including enzymes [26].

Unlike proteins, the fat content was lower in fruits stored at 25°C than in refrigerated fruits. The loss of fat content in control fruits was due to the softening, which occurs in the cell membrane by the loss of phospholipids during fruit ripening [27]. It could also be attributed to conversion of lipids to volatile compounds in the synthesis of aroma compounds [27]. A study with Annona cherimola L. found that weakening of the cellular membrane was associated with the loss of phospholipids. This effect was linked with high ethylene production rate of the fruits during ripening, which led to high enzymatic activity and cell membrane disruption [28]. In all refrigerated fruits (without or with 1-MCP), storage probably caused a decrease in the activity of enzymes that degrade the cell wall and the lipids content did not decrease.

Soluble sugars displayed significant differences between treatments (P < 0.05). Control fruits had 74.06 g/100 g DM, while storage at 16°C of soursop fruits, with or without emulsions, generated evident chilling injury symptoms such as browning, hard texture at touch, and abnormal ripening. The low soluble sugar content in the pulp of fruits stored at 16°C was probably caused by the reduction in starch degradation by a low oxidative capacity of the mitochondria [29] and also produced chilling injury symptoms. This coincided with other studies like that by Vishnu Prasanna et al. [30] who reported that the carbohydrate content of cherimoya decreased when fruits were stored at 10–15°C. With respect to 1-MCP, 1-MCP + ClO:90, and 1-MCP + F10:90 fruits, chilling injury symptoms were not observed and fruits ripened normally, but soluble sugars were statistically smaller (P < 0.05) than in control fruits (25°C) and greater than fruits that were stored at 16°C without 1-MCP. It could be caused by the synergistic effect of 1-MCP and emulsions applied [31].

3. Results and Discussion

3.1. Proximate Analysis. Table 1 shows that moisture content in fruits is composed mainly of metabolic enzymes; hence, protein concentration increased during ripening of fruits because numerous enzymes are synthetized. The low protein content found in the refrigerated fruit pulp may be attributed to decreased metabolic rate caused by the postharvest treatments and refrigerated storage. The soursop fruit showed a reduction in ethylene production rate [25] and probably had a decreased protein synthesis including enzymes [26].

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Ash content in fruits stored at 16°C was significantly different (P < 0.05) at all other treatments, and this result
coincided with the highest hardness values observed in the fruits. Regarding the greater mineral content in the fruits stored at 16°C with and without emulsions, it can be attributed to the alterations in the structure of cell wall polysaccharides and their covalent cross-links with minerals when they suffer chilling injury, which could maintain higher levels of calcium in the fruit with the formation of calcium pectates [32].

TDF content in Table 2 shows that fruits stored at 25°C had the lowest TDF content with 19.82 g/100 g DM, with 9.31 g/100 g DM of SDF and 10.49 g/100 g DM of IDF. In refrigerated fruits without 1-MCP (16°C, C10:90 and F10:90), TDF values had a range of 20.81–21.66 g/100 g DM, while in the fruits treated with 1-MCP or 1-MCP + emulsions, the results of TDF ranged 22.75–24.17 g/100 g DM, which was significantly higher than for all other treatments. TDF decreased in mature control fruit because of the activity of pectinmethylesterases, polygalacturonases, and cellulases. These enzymes catalyze the desterification and depolymerization of the polysaccharides that are part of SDF and IDF, such as pectin, hemicellulose, and cellulose in the cell wall [33]. Ramírez and Pacheco [34] reported TDF content in soursop of 49.34 g/100 g DM. However, this difference with respect to our results may be attributed to the analytical method and the soursop variety employed. TDF depended on the SDF and IDF proportion measured for each treatment. However, it was clear that fruits without 1-MCP at 16°C had lower SDF content. This is probably caused by abnormal hardening by chilling injury, which suggests decreased hydrolysis in the cell wall and, consequently, smaller values of SDF and greater values of IDF. Fruits treated with 1-MCP and 1-MCP + emulsions had greater amounts of TDF than control fruit and refrigerated fruit without 1-MCP. It is possible that these fruits had an increase in SDF caused by hydrolysis of cell wall material given that normally fruits were soft, but the hydrolysis was not in the same extent than control fruit, because the latter showed a higher content of IDF caused by the application of 1-MCP and edible coatings. These results coincided with firmness retention in fruits treated with 1-MCP and emulsions [6]. It has also been reported that either together or in separate applications 1-MCP and edible coatings can achieve the reduction in the activity of hydrolases or fruit softening [5, 35, 36].

3.2. Vitamin C. Vitamin C content in control fruit (25°C) was 40.56 mg/100 g FW (Figure 1). Fruits stored at 16°C, with or without emulsions had a statistically smaller (P < 0.05) vitamin C content than control fruits (25°C). In fruits treated with 1-MCP we found values of 45.24 mg/100 g FW; meanwhile in 1-MCP + C10:90 and 1-MCP + F10:90 fruits, vitamin C content values were 53.30 and 55.78 mg/100 g FW, respectively. The vitamin C content in control fruits was high even if compared to values reported by Barreca et al. [7] in soursop, who found a concentration of 3.30 mg/100 g FW. Fruit varieties employed and environmental conditions could explain the difference. In fruits stored at 16°C without 1-MCP it is possible that chilling injury contributed to reduce the synthesis of vitamin C; while in fruits stored at 16°C with 1-MCP, this compound could prevent chilling injury and at the same time delay the synthesis of enzymes that degrade ascorbic acid and also edible coatings acted synergistically with 1-MCP to reduce gas exchange and decrease the oxidation of vitamin C [37, 38]. Ma et al. [39] reported that the application of 1-MCP in broccoli decreased the gene expression of ascorbate peroxidase precursor synthesis, the main oxidative enzyme.

Table 2: Soluble (SDF), insoluble (IDF), and total dietary fiber (TDF) of seedless pulp obtained from soursop stored at 25°C, 16°C, fruits with emulsions and stored at 16°C; fruits treated with 1-MCP and stored at 16°C; fruits treated with 1-MCP + emulsions and stored at 16°C (g glucose/100 g dry matter of edible portion)*.

| Treatment                | SDF    | IDF     | KI       | TDF     |
|--------------------------|--------|---------|----------|---------|
| 25°C (control)           | 9.31 ± 0.96a | 4.93 ± 0.69d | 5.56 ± 0.48a | 19.82 ± 0.24a |
| 16°C         | 8.69 ± 0.27c | 6.64 ± 0.31c | 5.31 ± 0.41b | 21.66 ± 0.47b |
| C10:90      | 8.31 ± 0.26b | 8.51 ± 0.29b | 4.32 ± 0.70bc | 20.81 ± 0.61bc |
| F10:90      | 8.47 ± 0.95c | 8.45 ± 0.40b | 4.02 ± 0.77c | 21.45 ± 1.32b |
| 1-MCP       | 9.77 ± 0.11bc | 7.31 ± 0.33bc | 5.66 ± 0.10a  | 22.75 ± 0.15b  |
| 1-MCP + C10:90 | 8.97 ± 0.39b | 8.75 ± 0.13b | 4.79 ± 0.19b  | 23.80 ± 0.35b  |
| 1-MCP + F10:90 | 10.25 ± 0.08a | 8.83 ± 0.40a | 4.13 ± 0.10c  | 24.17 ± 0.98a  |

*Values are the mean ± SD (n ≥ 6). Different letters in columns indicate significant differences between samples using LSD test with α = 0.05. † Nonstarch polysaccharides.

![Figure 1: Vitamin C (mg ascorbic acid/100 g fresh weight of edible food) of seedless pulp obtained from soursop stored at 25°C, 16°C, fruits with emulsions and stored at 16°C; fruits treated with 1-MCP and stored at 16°C; fruits treated with 1-MCP + emulsions and stored at 16°C. Different letters indicate significant statistical differences between samples using the LSD test with α = 0.05.](image-url)
1-MCP + emulsions and stored at 16\(^\circ\)C; however, in all fruits stored at 16\(^\circ\)C, reported that application of 1-MCP decreased PPO activity, phenols, and tannins than control fruit [38,44]. It has been found that the decrease of tannins during ripening of orange fruit was caused by PPO, which turned tannins into simple phenols. The use of emulsions could reduce the exchange of oxygen and thus produce lower PPO activity and preserve tannins since fruits with emulsions had higher contents of CT. Waxes were used on lychee and pomegranate as a postharvest application and it was found that PPO activity decreased. Also, in these same fruits was found greater content of phenols and tannins than control fruit [38,44]. It has been reported that application of 1-MCP decreased PPO activity and preserved EP [45]. The effect of 1-MCP and emulsion combined applications on HP and CT has not been studied; however it is possible that both treatments act synergistically to decrease the synthesis and activity of enzymes that degrade these compounds.

3.4. Antioxidant Capacity (AC). The AC in control fruit was 76.07\(\mu\)mol TE/g DM (Table 3), while AC in fruits stored at 16\(^\circ\)C with or without emulsion AC was lower (72.29–75.65\(\mu\)mol TE/g DM). AC increased in fruits treated with 1-MCP + emulsions and was highest in 1-MCP + Fl10:90 (89.84\(\mu\)mol TE/g DM). 1-MCP and 1-MCP + emulsion treatments preserved vitamin C and total polyphenols in fruits; therefore greater AC was possible [46]. The EP of each treatment showed a high correlation (\(r^2 = 0.9606–0.9704\)) with the AC, indicating that the EP contained in soursop had the ability to reduce metals such as iron (Fe); however vitamin C showed a low correlation (\(r^2 = 0.4969–0.4956\)). The correlations found between PE and AC agree with reports by other authors [47]. High AC is likely because EP have a large number of hydroxyl (OH) functional groups in their chemical structure, which are responsible for conferring its high antioxidant power. Gil et al. [48] found no correlation between vitamin C and the AC determined by the FRAP or ABTS assays in nectarines, peaches, and plums. Thus we propose that the AC of vitamin C is largely dependent on the amount originally present in the fruit.

### Table 3: Extractable polyphenols (EP), hydrolysable polyphenols (HP), condensed tannins (CT), total phenolic content (TPC) (GAE/100 g dry matter of edible food), and ferric reducing antioxidant power (FRAP, \(\mu\)mol TE/g dry matter of edible food) of seedless pulp obtained from soursop stored at 25\(^\circ\)C, 16\(^\circ\)C, fruits with emulsions and stored at 16\(^\circ\)C; fruits treated with 1-MCP and stored at 16\(^\circ\)C; fruits treated with 1-MCP + emulsions and stored at 16\(^\circ\)C.

| Treatment               | EP     | HP     | CT     | TPC    | FRAP    |
|-------------------------|--------|--------|--------|--------|---------|
| 25\(^\circ\)C (control) | 1.14 ± 0.04\(^a\) | 0.25 ± 0.01\(^c\) | 1.16 ± 0.03\(^d\) | 2.55 ± 0.02\(^c\) | 76.07 ± 1.08\(^b\) |
| 16\(^\circ\)C            | 0.99 ± 0.01\(^c\) | 0.28 ± 0.01\(^b\) | 1.34 ± 0.13\(^b\) | 2.61 ± 0.05\(^c\) | 72.29 ± 1.03\(^c\) |
| C10:90                  | 1.05 ± 0.01\(^b\) | 0.37 ± 0.01\(^b\) | 1.51 ± 0.12\(^b\) | 2.93 ± 0.04\(^b\) | 74.59 ± 2.82\(^b\) |
| F10:90                  | 1.00 ± 0.05\(^c\) | 0.34 ± 0.07\(^bc\) | 1.57 ± 0.03\(^b\) | 2.91 ± 0.05\(^b\) | 75.65 ± 1.36\(^b\) |
| 1-MCP                   | 1.00 ± 0.03\(^c\) | 0.40 ± 0.60\(^ab\) | 1.59 ± 0.02\(^b\) | 2.99 ± 0.55\(^ab\) | 83.50 ± 0.47\(^a\) |
| 1-MCP + C10:90          | 1.37 ± 0.04\(^a\) | 0.42 ± 0.09\(^a\) | 1.83 ± 0.03\(^a\) | 3.62 ± 0.05\(^a\) | 87.37 ± 9.67\(^a\) |
| 1-MCP + F10:90          | 1.29 ± 0.07\(^a\) | 0.44 ± 0.06\(^a\) | 1.79 ± 0.03\(^a\) | 3.52 ± 0.04\(^a\) | 89.84 ± 0.72\(^a\) |

*Values are the mean ± SD (n ≥ 6). Different letters in columns indicate significant differences between samples using LSD test with α = 0.05.

3.3. Total Phenolic Content. The effect of postharvest treatments was significant (\(P < 0.05\)) for total phenolic content (Table 3). Control fruits showed a total phenolic content of 2.55 g GAE/100 g DM; however, in all fruits stored at 16\(^\circ\)C the amount of total polyphenols was higher than control fruit. In fruits maintained at 16\(^\circ\)C, we found 2.61 g GAE/100 g DM while in C10:90 and F10:90 fruits the CT and HP content was slightly higher than fruits at 16\(^\circ\)C; therefore, total phenolic content was 2.91–2.93 g GAE/100 g DM. Fruits with 1-MCP and 1-MCP + emulsions application retained higher values of total phenolics in all treatments; however the 1-MCP + emulsions (candelilla or flava) treatments preserved the highest content of EP, CT, and HP and at the same time increased shelf life. Barreca et al. [7] reported a polyphenol content of 0.054 g GAE/100 g DM in soursop, which did not coincide with the data of total polyphenolic content found in our experiments for control fruits, because the former authors quantified EP only. The lowest content of EP and HP in fruits at 16\(^\circ\)C with and without emulsions was related to intense browning in the pulp caused by chilling injury. Lima de Oliveira et al. [42] reported that this physiopathy in soursop occurred by the oxidation of phenolic compounds because of polyphenoloxidase (PPO) activity. Adetuyi et al. [43] found that the decrease of tannins during ripening of orange fruit was caused by PPO, which turned tannins into simple phenols. The use of emulsions could reduce the exchange of oxygen and thus produce lower PPO activity and preserve tannins since fruits with emulsions had higher contents of CT. Waxes were used on lychee and pomegranate as a postharvest application and it was found that PPO activity decreased. Also, in these same fruits was found greater content of phenols and tannins than control fruit [38,44]. It has been reported that application of 1-MCP decreased PPO activity and preserved EP [45]. The effect of 1-MCP and emulsion combined applications on HP and CT has not been studied; however it is possible that both treatments act synergistically to decrease the synthesis and activity of enzymes that degrade these compounds.
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