Effect of storage condition on color, vitamin C content, polyphenol content and antioxidant activity in fresh soursop pulp (*Annona muricata L.*)

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Abstract *Annona muricata Linn.* (soursop) belongs to the Annonaceae family. This plant has been traditionally used for the treatment of various infectious and inflammatory diseases. In this study, the effect of storage conditions (room and cold condition) on *Annona muricata* nutrients was evaluated on the basis of color, vitamin C, polyphenol content and antioxidant activity (DPPH). The change in Lab brightness (64.34 ± 4.18a, -4.61 ± 0.31a, 12.80 ± 0.57a for fresh sample) was negligible during the 10 day cooling process (66.22 ± 2.33ab, -0.58 ± 7.89a, 9.03 ± 0.85b). These criteria have not changed compared to the original sample after 2 days. The effect of room temperature on properties of Soursop was significant. After 8 and 10 days, it was impossible to quantify TAA, TPC and ABTS of the sample. The values of the two samples (8 and 10 days) at low temperatures were respectively 4.46 ± 0.35 and 3.27 ± 0.33 (TAA); 3.00 ± 0.05 and 2.64 ± 0.30 (TPC); 0.66 ± 0.01 and 0.69 ± 0.04 (free-radicals scavenging capacity). The appearance and morphology of the samples are also graphically described.

1. Introduction

Fruits and vegetables provide an abundant mix of antioxidants, such as Vitamin C, polyphenols and carotenoids [1-3]. In recent years, they have received much of attention since many epidemiological researches suggest that consumption of polyphenol rich fruits is associated with a reduced risk of heart disease, cancer, and brain dysfunction [4-7]. Chemical compounds including sodium benzoate, nitrite, and sodium have been examined and determined safe for food preservation.

Finding appropriate preservation methods for different fruits have been receiving a great deal of public attention due to the loss of nutrients in fruits after harvest [8]. *Annona muricata* (namely soursop) belong to the Annonaceae family which cultivated mainly in tropical and subtropical countries rich in Vitamin C, Phenolics compounds, thiamin, arginine and lysine, glutamic acid, aspartic acid, glycine-serine, antioxidant compounds [9,10]. These groups of bioactive substances plays a vital role for the body which prevent the formation of free radicals [11].
Compounds that can free-radicals scavenging activity lose large amounts through fruit ripening and oxidation. Therefore, the change or loss after ripening of fruit should be noted storage time at 6 levels: 0-2-4-6-8-10 days, the loss of nutrient components will be measured at each level. Low temperatures control inactivation of ripening enzymes and decomposition of naturally beneficial compounds [12]. Cold storage aid to maintain fresh food over long periods which reduces the minimal loss of nutrients such as antioxidant content or vitamins [6]. In some previous studies about the condition of preserving fruits that have not been tested on soursop. Therefore, the present study was carried out to the effects of the soursop pulp on different storage period maintained at 1°C temperature on total ascorbic acid, total polyphenol, and antioxidant activity and color changes.

2. Methods and materials

2.1. Sample preparation
Soursop was purchased from the Tan Phu Dong market in Vietnam. This work was aimed under studying its storage stability at refrigeration (1°C) and ambient (30°C) temperatures.

2.2. Determination of Total Ascorbic Acid content
Vitamin C content in the sample is determined based on DCPIP titration method which was previously described by Manas Denre [13]. Based on ascorbic acid oxidation with 2,6 dichlorophenolindophenol acid (DCPIP) into dehydroascorbic acid and colorless lenco derivatives. The optimized reaction at pH between 3 and 4 redundant DCPIP drops will cause the solution to turn pink. Ascorbic acid content is calculated based on two equations:

\[ TAA_1 \left( \frac{mg}{g \, WM} \right) = \frac{(V_1 - 0.05) \times V_2 \times 10 \times m_1 \times df}{10 \times m_2 \times (V_3 - 0.05)} \]  
\[ TAA_2 \left( \frac{mg}{g \, DM} \right) = \frac{TAA_1 \times m_2}{m_3} \]

Where:
- \( V_1 \) is the average DCPIP volume of the sample (ml)
- \( V_2 \) is the volume of the container of the extracted sample (ml)
- \( m_1 \) is the standard mass of ascorbic acid (g)
- \( df \) is the sample dilution factor
- \( m_2 \) is the volume of fresh samples analyzed (g)
- \( V_3 \) is the DCPIP volume of ascorbic acid standard (ml)
- \( m_3 \) is the sample mass according to the dry matter concentration (g)

2.3. Determination of Total Phenolics Content
Total phenolic contents in pulp extract were evaluated using Folin–Ciocalteu’s colorimetric assay, as modified by Sinanoglou [14]. Absorbance was measured at room temperature at 765 nm with a equipment (Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100g dry weight, using a standard curve with 2-10 mg/L gallic acid (y = 0.01697x + 0.02266, R\(^2\) = 0.99951).

2.4 Determination of antioxidant capacity by DPPH
Antioxidant capacity was measured by scavenging free radical (DPPH) which was performed based on the method described by Ngoc at el [15]. Antioxidant compounds had the ability to scavenge free radicals so it discolor purple in DPPH solution. The sample (2-gram) was ground and extracted with
ethanol absolute. The extract (500µL) was mixed with 2500µL DPPH solution. The sample was kept in the dark about 60 minutes and then measuring absorbance at 517 nm (using Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer). The result was expressed by mg of Ascorbic Acids equivalent per gram of dry matter (mg AA/g dry matter).

The calibration curves value for DPPH:

\[ y = 15.617x - 24.887 \quad R^2 = 0.99998 \] (3)

2.5. Determination of Lab* parameters
The color lightness of the sample is measured based on measuring and analyzing color of food surfaces [16]. Lab* model has the largest gamut encompassing all colors in the RGB (red, green, and blue) and CMYK gamuts (cyan, magenta, yellow, black). The sample is selected at three random positions on the surface, the colorimeter returns the results of three parameters for each position. A device used to measure color is 0.3NH Scanner Chroma colorimeter (NR60CP model).

2.6. Data analysis
The results obtained were analyzed statistically using Statgraphics Software. The mean total phenol content and antioxidant activity of the samples were compared using one-way ANOVA followed by LSD post hoc multiple comparisons. Statistical significant difference was performed at \( \alpha = 0.05 \).

3. Result and discussion

3.1. Effect of storage conditions on the color and properties of soursop
Table 1 illustrates the effect of time on the color and properties of soursop. Results showed that 2 days samples in room conditions appeared yellow mold and were increased in 4 days samples. Microbial contamination causes difficulty in surface color measurement, so they are not presented in table 2. Besides, the appearance of non-normal odor at two samples was also recorded. To explain this failure, Rawat 2015 and Viana at el 2005 showed that microorganisms are available in the air to penetrate and grow at the surface of the material, This exposure increases as the sample has available nutrients (carbon, energy) to provide for their life and metabolism [17]. However, two samples with storage time at 1°C were similar to fresh samples, smooth surfaces, tight fiber structure, initial aroma.

| Fresh Day number | 2 day | 4 day | 6 day | 8 day | 10 day |
|------------------|-------|-------|-------|-------|-------|
| RC               |       |       |       |       |       |
| Cold Storage     |       |       |       |       |       |

| RC               | None  | None  | None  |
| Cold Storage     |       |       |       |

This changes insignificantly when increasing storage time. Spoilage phenomenon appears around the outer shell and changes the center color. This is explained in the report of Cano and Koutsoumanis that temperatures lower than 5°C strongly inhibit peroxidase, phylophenol oxidase enzyme activity and narrow the Log phase of microorganisms, especially mold [18].
Table 2. Lab\* value of normal preservation and nutritional value of cold storage samples over 10 days

| Sample    | L\*  | a*  | b*  | TAA         | TPC          | DPPH        |
|-----------|------|-----|-----|-------------|--------------|-------------|
| Fresh     | 64.34 ± 4.18a | -4.61 ± 0.31a | 12.80 ± 0.57a | 9.05 ± 0.00a | 5.85 ± 0.09a  | 1.67 ± 0.03a  |
| 2 day     | 69.47 ± 4.66abc | -4.70 ± 0.57a | 12.41 ± 2.00a | 6.65 ± 0.82b | 4.36 ± 0.16b  | 1.02 ± 0.02b  |
| 4 day     | 71.89 ± 0.66b  | -4.82 ± 0.62a | 12.33 ± 1.01a | 5.51 ± 0.41b | 4.07 ± 0.01b  | 0.84 ± 0.00c  |
| 6 day     | 71.20 ± 2.03de | -5.00 ± 0.78a | 12.60 ± 0.82a | 3.33 ± 0.82c | 3.87 ± 0.08c  | 0.88 ± 0.01c  |
| 8 day     | 70.72 ± 2.03de | -5.39 ± 0.12a | 10.34 ± 0.82b | None         | None         | None         |
| 10 day    | 66.22 ± 2.33ab | -5.58 ± 7.89a | 9.03 ± 0.85b | None         | None         | None         |

* Means followed by same letter do not differ by the Duncan test (p < 0.05). ¹ room conditions sample. ² Dry matter.

The color and nutrient content, TAA, TPC and free-radical scavenging activity (DPPH) of pulp are reported in table 2. The CIE L*a*b* color system displays a statistically difference between fresh and preserved samples. The a* of fresh samples has a value of -4.61 ± 0.31a within the gamuts of green trend similar to 10 days value -5.58 ± 7.89a in low temperature conditions. The L* value increased significantly from 64.34 ± 4.18a to 71.20 ± 2.03de at 6 days and approximately 8 days. Although the temperature has the effect of limiting the activity of browning enzymes, the process of darkening the surface still occurs but at a slow rate. Similar to the reduction of biological color compounds in the record of Oey at el [19].

The total content of substances with the antioxidant activity of stored samples at room temperature is also presented in table 2. This result limits the presentation of samples 8 days and 10 days, this is caused by failure to analyze the putrefy sample. The TAA reduction had a difference between the original sample 9.05 ± 0.00a mg/g DM and after 2-4 days (there was no significant change during this period). Vitamin C degradation continued until day 6, when TAA kept about 36.80% of the sample that could not be used under normal conditions. Enzyme activity affects this reduction, TAA content is inversely proportional to the shelf-life plant [20].

The trend of decreasing gradually over time applies to free radical operations TPC and DPPH. There is a linear correlation with these two values. The control sample with TPC is 5.85 ± 0.09 mg GAE/g DM similar to the result of Eloy at el 2017 [21] and tend to decrease when processing time increases. The negligible decrease from day 2-4 is similar to TAA content. The absorbance measurement in the Folin- Ciocalteu assay is affected by the oxidation-reducing reaction that TAA creates. In addition, antioxidant activity (DPPH) is also affected, decreasing 0.65-0.83 (mg AA/g DM) on days 2 and 4, respectively.

Figure 1 shows that the content of Vitamin C and TPC in cold soursop varies over time. As the results show, the distribution of these two indicators through the survey days is the same. 9.05 ± 0.00 (fresh) decreased 3.42mg/g DM remaining 5.63 ± 0.21 (4 days) of TAA value which is in accordance with the law of degradation of Vitamin C [22]. In previous report, surface contact with light, air and high humidity increased the loss of TAA[22]. Although significantly reduced, this content is thought to be higher than the storage method at room temperature. TAA retention after finishing preservation (at 10 days storage) achieved 36.13% (which continues to decrease 5.94 ± 0.41 to 4.46 ± 0.35 at 8 days). Lower than 10°C there is a loss of TAA but the speed is limited compared to the RC sample [23].

Same principle, TPC presented in figure 1 decreased from 5.58 ± 0.09 to 4.84 ± 0.05 at the second day of storage and changed insignificantly over 6 days. It is possible that some enzymes polyphenols oxidase are restricted from activity at temperatures below 5°C [24], TPC reduced 3.21mg/g DM during 10 days of cold storage.

In general, TAA and TPC content decreased during storage. Every 2 days, Vitamin C content
decreases significantly. TPC fell after 2 days and stabilized for the next 4 days. Moreover, this data has a more positive meaning to preserving in room conditions.

![Figure 1](image)

**Figure 1.** TAA (mg/g DM) and TPC (mg GAE/g DM) in cold storage Soursop pulp

3.2. *The antioxidant capacity is affected during cold storage*

According to figure 2 data the antioxidant capacity decreased insignificantly from 1.17 ± 0.04 to 1.05 ± 0.03 with pulp and 2 days, respectively. Overall, every 4 days of storage, this content decreased significantly compared to the original sample. The values of free radical resistance in samples 4 and 6 days did not differ by 1.15 ± 0.02 and 1.04 ± 0.05, respectively, although there was a significant decrease compared to the previous and the next samples. 0.35 mg/g DM free radicals capacity from day 6 to 10. Total preservation time, DPPH value decreased by 41.03%. The percentage of nutrient loss and antioxidant capacity depends on the type of material, the structure of the product [25] or in the difference in solvent extraction. Changes during storage of antioxidation compounds are also described in previous reports on apples and oranges [26, 27].

![Figure 2](image)

**Figure 2:** Loss of oxidation activity over time: 0-10 days

4. **Conclusion**

Nowadays, using medicinal plants has been receiving a great deal of public attention in clinical and therapeutic potential. *A. muricata* play an essential function in traditional medicine such as stomach pain, hypertension, diabetes, and so on. This research investigated and revealed that Soursop is stored at low temperatures (1°C) keeping TAA (36.13%), TPC (45.13%), antioxidant activity (58.97%)
higher than the room environment. Moreover, colors and sensory description are better. It can be concluded that the sample preserves 2 cold days almost as the original sample. However, the values of interest are reduced when the processing time is increased. Cold storage time needs to be adjusted more broadly and surveyed storage temperature.

5. References

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