Studies on Polyphenol content, flavonoid content, and antioxidant activity of Graviola tea pulp (Annona muricata L.)

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Abstract. Tea is the most commonly consumed beverage worldwide due to promote health and prevention of some human diseases. Soursop tea pulp is well-known for containing a significant content of ascorbic acid (TAA), polyphenols (TPC) and flavonoids (TFC). In the present study, different pulp tea samples with varying dates of production were checked for TAA, TPC, TFC, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activities, followed by sensory evaluation. There is a difference in TAA between sample (1) and (4), achieved 7.43 ± 0.04 mg/g DM and 12.21 ± 0.04 mg/g DM, respectively. TFC content ranged between 13.10 and 18. The phenolics group in tea is high and there are differences between the 3 recent production samples with (1): 3.81mg QC/g DM fluctuation. In sample 1, the antioxidant activity of the ethanol extract of Soursop was correlated with total phenolic and flavonoid content with values of 0.26 mgAA/g DM, 2.61 mgAA/g DM for DPPH and ABTS scavenging activity, respectively. Moreover, the CIE Lab* color space of the soursop tea is measured in the dark area, the L* difference achieved at 30.82 ± 1.24 (2) lowest and the highest 37.42 ± 4.77 (1). Consumer sensory evaluation is performed with results based on a 5-point scale describing: flavor, aroma, color, and favorite. The results showed sensory acceptance of all four samples and negligible difference in scores.

1. Introduction
In recent years, the primary concern of consumer is nutritional values, which leads to the requirement for research on healthy compounds, such as organic antibacterial, anthocyanin content groups, or some bioactive compound [1,2]. Soursop (Annona muricata L.) nectar plays an important potential for the international market which cultivated in tropical countries such as Vietnam, Thailand, Africa, Malaysia and the United States. Soursop fruit is highly perishable by polyphenol oxidase enzyme which affected their sensory and nutritional qualities. In Soursop fruit contains many vitamins (especially Ascorbic Acid and Thiamin), abundance of free amino acids, antioxidant capacity, glycine, serine, citrulline, cystein, antioxidant activity. Oxidation process happens continuously, creating a premise for aging and health degradation. Free radicals are generated unexpectedly and their negative impacts [3,4].

Food industries require food source which provides active ingredients to resist free radicals. The tea-making process, like withering, bruising, drying, causes significant damage to antioxidant components such as TPC, TFC, TAA, and free-radical scavenging activity [5,6]. However, this process also has different advantages of preservation and commercial. In recent studies, flavonoid in black tea and green tea, phenolics content in Bosnian Crataegus Species, antioxidation activity in oolong tea [7,8] proved that the
values of TPC, TFC are reduced. Soursop leaves are widely commercialized as tea and functional foods, and medicinal properties are also evaluated; Extracted seed ingredients are recovered for application to pharmaceuticals and pesticides. Soursop tea pulp provides a novel approach to herbal tea consumer products. Studies should focus on evaluating the quality of this product. The reason for the effect may depend on the area of cultivation as well as the climatic conditions, and harvesting methods. Researcher reports that the composition and biological activity in Soursop tea pulp is limited. This study aims to present the determination results of various quality indicators including ascorbic acid, antioxidant activity, polyphenol and flavonoids content of the leaves of four Siamese custard tea samples that differ in production dates.

2. Materials and methods
2.1. Chemicals and reagents
2,6-dichlorophenolindophenol (DCPIP) was purchased from India. Analysis agents including Folin-Ciocalteu Reagent (FCR), Gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were commercially obtained from Sigma-Aldrich (US). Other chemicals including methanol, Na2CO3, L-ascorbic acid NaHCO3 had the purity of at least 99.5% and were purchased from China.

2.2. Plant Material
Graviola tea pulp were collected from Tan Phu Dong, Tien Giang Province, Vietnam. Four samples with production date: March 29, April 22, April 25 and April 27 were selected and numbered as 1, 2, 3 and 4, respectively. The samples were marked with the date of production and indicator analysis. The sample (2 g) was ground and extracted with ethanol. The afford extract was kept at ambient temperature for 30 min before being subjected to filtration through Whatman No.1 paper.

2.3. Determination of total polyphenol content (TPC), total flavonoid content (TFC) and antioxidant activity
The titration method by DCPIP is described previously by Manas Denre [9] based on the reaction to form dehydroascorbic acid and colorless lenco derivatives of oxidation of Vitamin C with 2,6 dichlorophenolindophenol. Procedure for determination of TPC was in accordance with Chandra et al in which Folin-Ciocalteu colorimetric methods were employed with gallic acid being as a standard [10]. Base on the aluminum chloride colorimetric method, the TFC was determined [11]. The antioxidant activity of Soursop tea pulp was tested using DPPH assay [12] and free-radical scavenging by ABTS assay [13]. Determination of Lightness: The CIE Lab* color space is reference color preference based on three values L*, a* and b* [14].

2.4. Sensory evaluation
The survey results were recorded from the non-training evaluation committee. A 5g sample diluted with boiling water (1:200 ratio), with 20ml for each tasting. Three-digit encoded samples avoid being affected by user senses. The appropriate table is based on the given score corresponding to the standard description term (TCVN 3215 - 79 test).

Table 1: The term sensory description of graviola tea

| Critical coefficient | Flavor | Color | Aroma | Favorite |
|----------------------|--------|-------|-------|----------|
| 5                    | taste cool, tea flavor, enjoy delicious | Pellucid, bright yellow, typical for tea | featured for custard apple | Like extremely |
| 4                    | taste cool, enjoy delicious | Pellucid, slightly yellow | lightly | Like |
| 3                    | tea flavor | Cloudy yellow | Aromatic, not characteristic | Accept |
| 2                    | acrid, enjoy delicious | Not typical tea color | odorless | Neither like nor dislike |
| 1                    | acrid, enjoy poorly | strange color | strange smell | Dislike |
2.5. Statistical Analyses
Experiments are performed triplicate. To confirm differences in results between experiments, SPSS 15.0 software (SPSS Inc., Chicago, USA) was used to perform analysis of variance (ANOVA). Results are expressed as mean value ± standard deviations and differences were considered significant at $p < 0.05$.

3. Results and discussions

3.1. Total polyphenol and total flavonoid contents
Figure 1 illustrates the content of phenolics and the ability of free radical capture by ABTS method. It was found that there was a similarity in the concentration of TPC content in the four samples tested. The highest TPC content was seen in the sample (4), achieved $8.83 \pm 1.33b$. The level TPC of sample (3) was slightly higher than the sample (2), with $7.80\pm0.22$ and $7.55\pm0.11$, respectively. The lowest TPC was seen in the sample (1) at $5.02 \pm0.64$. ABTS at all sample achieved around 26.6 µg GAE/mg extract. This results is in line with a previous study which proved that the catechin and the aflavins significantly contribute to total TPC content even before and after processing [15]. The concentration of dry matter increases with the loss of moisture, causing the ABTS scavenging ability in the Soursop to increase.

![Figure 1: Total phenolic content and ABTS scavenging activity of soursop tea pulp](image1)

![Figure 2: TFC and DPPH scavenging ability by DPPH of soursop tea pulp](image2)

Figure 2 reports the value of TFC and the ability of oxidation resistance by DPPH. Here, the sample 1 for biological activity (flavonoids) achieved $0.14 \pm 0.01$, almost no difference between the remain samples. Moreover, the DPPH of sample (4) achieved $0.33 \pm 0.01$, which is higher than the remaining values samples. The previous study shows the effect of harvesting stage to the antioxidant value of soursop, which indicates significant differences in the content of (1) and (2). Moreover, the degradation of these active substances is influenced by the ripening process of the fruit.
The tea pulp TAA content showed in Figure 3. Notice that there is a significant difference in the first sample value: 7.43 ± 0.04 compared to the remaining sample (higher than ~0.99, 5.61, 4.47 mg/g DM of (2), (3) and (4), respectively). As mentioned above, the production date of each sample was different. It can be concluded that the content depends on the crop, cultivation area and during processing [16]. Non-vacuum packaging reduces the significant amount of TAA by the air inside.

3.2. Evaluation of sensory and colorimeter.

Table 2 illustrates the color value of the four tea samples. The L* index shows the dark space and the value of the higher browning effect. The sample (2) has the lowest 30.82 ± 1.24 (L*), values a * and b * tend to approach the center of three-dimensional space. Based on that, the total difference color (TCD) is calculated based on the difference between the product and fresh pulp. Notice that the color difference of samples (1), (3) and (4) is from 20.71 to 30.62; (2) has the highest difference of 36.31. It is understood that Graviola tea colors depend on the original input and also parameters of processing (temperature and time). The previous study shows that color change in tea production was massive and sought a way to improve this significant index [17].

Table 2: Lightness of prickly custard apple tea

| Sample | (1)          | (2)          | (3)          | (4)          |
|--------|--------------|--------------|--------------|--------------|
| L*     | 37.42 ± 4.77a| 30.82 ± 1.24b| 36.64 ± 1.11a| 36.73 ± 0.31a|
| a*     | 1.89 ± 0.13a | 2.44 ± 0.06b | 2.44 ± 0.12b | 3.21 ± 0.10c |
| b*     | 10.74 ± 0.89a| 10.11 ± 0.52a| 12.25 ± 0.45b| 12.39 ± 0.25b|

Aroma and other sensorial characteristics such as flavor and coloring determine overall sensory qualities of the product. The assessment results are presented in Figure 4, including the listed criteria and consumer preferences. Based on the flavor column chart, this result (ranging from 4-5/5) indicates that the tester can identify the taste (taste fresh, enjoy deliciously). Moreover, the sample (1) has the highest preservation time with a characteristic and more recognizable taste. Similarly, in term of color, nearly 4/5 points describe prickly custard apple-colored tea that is light yellow and non-significant differences between samples. However, the scores for odor compounds achieved at 3-4/5 points, and volatile aromatic components have low score. The evaluation of consumers shows that the samples (1) and (4) are similar. Previous studies show that the sip frequency affects the sensory results, along with the rapid changes in time of the compounds in tea, the sensory index shows the relative level of the sample [18]. The total score with an essential factor achieved greater than 11.2. Therefore, the product is capable of good commercialization.
4. Conclusions

The study of bioactive compounds in soursop tea pulp was determined. Values for Vitamin C content, total phenolic content, flavonoids content were significantly lost during processing. In this study, Pulp tea samples with different dates of production checked for TAA, TPC, TFC, antioxidant, and sensory evaluation. There is a difference in TAA between sample (1) and (4), achieved 7.43 ± 0.04 mg/g DM and 12.21 ± 0.04 mg/g DM, respectively. TFC content ranged between 0.13 and 0.18.

It was also found that total phenolic and flavonoid content largely determine the antioxidant activity of the ethanolic extract of Soursop. DPPH and ABTS scavenging activity was 26 mgAA /g DM and 2.61 mgAA /g DM, respectively.

The heterogeneity of input materials of product suppliers makes it difficult to identify changing trends of indicators. Moreover, the gap between production stages needs to be stabilized.

Acknowledgment
This study was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

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