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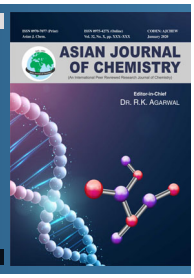

Studies on Polyphenol Content, Flavonoid Content and Antioxidant Activity of Graviola Tea pulp (*Annona muricata* L.) from Tien Giang Province, Vietnam

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Tea is the most commonly consumed beverage worldwide due to promote health and prevention of some human diseases. Soursop tea pulp is one of a new product focused on the values of total ascorbic acid (TAA), total polyphenol content (TPC) and total flavonoid content (TFC). A study using DPPH and ABTS method to detect the maximum of active ingredients in tea simultaneously. Different pulp tea samples with varying dates of production checked for TAA, TPC, TFC, antioxidation 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. The phenolics group in tea is high and there are differences between the 3 recent production samples with (1): 3.81 mg 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 IC_{50} of 0.26 mgAA/g DM, 2.61 mgAA/g DM for DPPH and ABTS scavenging activity, respectively. Moreover, the Lab* colour 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: flavour, aroma, colour and favourite.

Keywords: Total phenolic content, Total flavonoid content, DPPH, ABTS, Soursop (*Annona muricata* L.), Graviola tea pulp.

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 compounds [1,2]. The 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 ascorbic acid (TAA), total polyphenol content (TPC) and total flavonoid content (TFC) 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, antioxidant activity in

oolong tea [7,8] proved that the values of TPC, TFC are reduced. 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. The present study was carried out to evaluate TAA, total polyphenol, flavonoids content and antioxidant activity of the leaves of four Siamese custard tea samples that differ in production dates.

EXPERIMENTAL

2,6-Dichlorophenolindophenol (DCPIP) was imported from India. Other chemicals such as distilled water (with pH between 6.5 and 8), methanol (99.5% purity), Na_2CO_3 (99.5% purity), L-ascorbic acid (99.7% purity), $NaHCO_3$ (99.5% purity) were originated from China. 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 bought at Sigma-Aldrich Chemie, Co Ltd (USA).

Graviola tea pulp were collected from Tan Phu Dong, Tien Giang Province, Vietnam. Includes 4 samples with production date: March 29 (1), April 22 (2), April 25 (3), April 27 (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 extract was placed at room temperature about 30 min and then filtered through Whatman No.1.

Total polyphenol content (TPC) and total flavonoid content (TFC) and antioxidant activity: The titration method by DCPIP is described previously by Manas [9] based on the reaction to form dehydroascorbic acid and colourless lenco derivatives of oxidation of vitamin C with 2,6-dichlorophenol-indophenol. TPC was determined Folin-Ciocalteu colorimetric methods using gallic acid as a standard and being described by Visavadiya and Narasimhacharya [10]. Based 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* colour space is reference colour preference based on three values L*, a* and b* [14].

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

Statistical analyses: Experiments are performed triplicate. Statistical analysis was determined by SPSS 15.0 (SPSS Inc., Chicago, USA). Variance analysis (ANOVA) was applied to determine the differences between the experimental. Results are expressed as mean value ± standard deviations and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Total polyphenol and total flavonoid contents: Fig. 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 in the four samples tested. The highest TPC was seen in the sample 4, achieved 8.83 ± 1.33b. The level TFC of sample 3 was slightly higher than the sample 2, with 7.80 ± 0.22 and 7.55 ± 0.11, respectively. The lowest TFC was seen in the sample 1 at 5.02 ± 0.64. ABTS at all sample was 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.

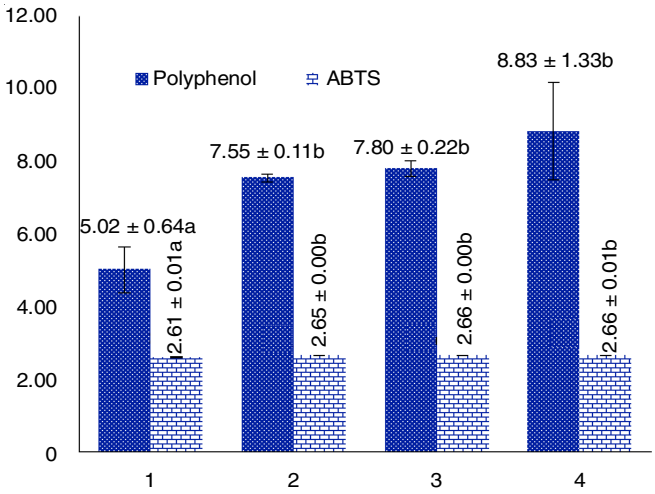

Fig. 1. Total phenolic content and antioxidation activity by ABTS of guanabana tea pulp

Fig. 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 ± 0.01, almost no difference between the remain samples. Moreover the DPPH of sample (4) achieved 0.33 ± 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 G1 and G2. Moreover, the

TABLE-1
TERM SENSORY DESCRIPTION OF *Graviola* TEA

Critical coefficient	Flavour	Colour	Aroma	Favourite
5	Taste cool, tea flavour, enjoy delicious	Pellucid, bright yellow, typical for tea	Featured for custard apple	Extreme like
4	Taste cool, enjoy delicious	Pellucid, slightly yellow	Lightly	Like
3	Tea flavour	Cloudy yellow	Aromatic, not characteristic	Accept
2	Acrid, enjoy delicious	Not typical tea colour	Odorless	Neither like nor dislike
1	Acrid, enjoy poorly	Strange colour	Strange smell	Dislike

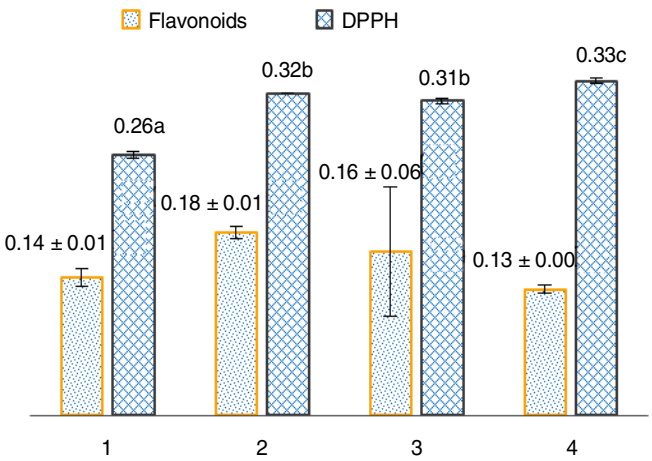

Fig. 2. Total flavonoid content (TFC) and free-radical scavenging ability by DPPH affected by harvest date

degradation of these active substances is influenced by the ripening process of the fruit.

Ascorbic acid content present in tea pulp is shown in Fig. 3. 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, 4.47mg/g DM of samples 2, 3 and 4, respectively). As mentioned above, the period of production to survey is uneven, namely 34-10-7-5 days. 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 ascorbic acid by the air inside.

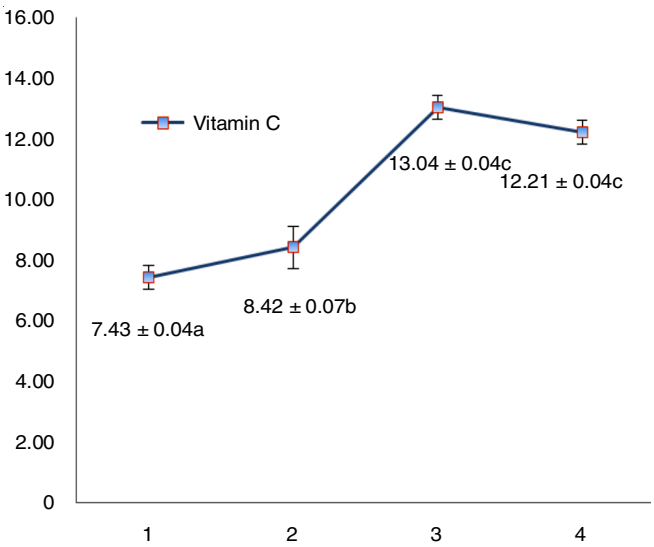

Fig. 3. Differences in total ascorbic acid (TAA) content (mg/g DM)

Evaluation of sensory and colorimeter: Table-2 illustrates the colour 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. The similar colour system is equal in samples (66.33 ± 3.32, -4.62 ± 0.31 and 12.80 ± 0.57 corresponding to L*, a and b* respectively). Based on that, the total difference colour (TCD) is calculated based on the difference between the product and fresh pulp. Notice that the colour 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 colours depend on the original input and also parameters of processing (temperature and time). The previous study shows that colour change in tea production was massive and sought a way to improve this significant index [17].

The sensory qualities of tea depend on aroma, as well as other sensory attributes such as flavour and colour. The assessment results are presented in Fig. 4, including the listed criteria and consumer preferences. Based on the flavour 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 colour, nearly 4/5 points describe prickly custard apple-coloured 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 statistics of favourite are equal in all samples, the total score with an essential factor achieved greater than 11.2. Therefore, the product is capable of good commercialization.

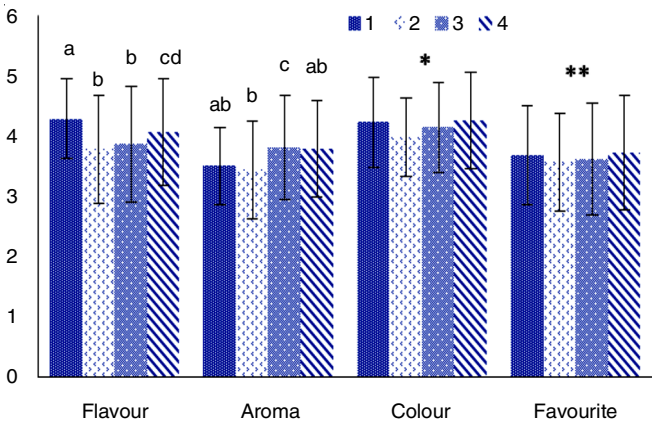

Fig. 4. Record transcripts from the tester's description (*, ** means of non-significant difference < 0.05)

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

Conclusion

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 total ascorbic acid (TAA), total polyphenol content (TPC) and total flavonoid content (TFC), antioxidation and sensory evaluation. There is a difference in total ascorbic acid between samples 1 and 4, achieved 7.43 ± 0.04 mg/g DM and 12.21 ± 0.04 mg/g DM, respectively. Total flavonoid content (TFC) content ranged between 0.13 and 0.18. The antioxidant activity of the ethanol extract of soursop was correlated with total phenolic and flavonoid content with values IC_{50} of 0.26 mgAA/g DM, 2.61 mgAA/g DM for DPPH and ABTS scavenging activity, 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.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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