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EFFECT OF STEAM BLANCHING AND CONVECTION DRYING CONDITIONS ON COLOR, VITAMIN C, TOTAL PHENOLIC CONTENT, FLAVONOID CONTENT AND ANTIOXIDATION ACTIVITY IN SOURSOP (*ANNONA MURICATA L.*) LEAF TEA

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ABSTRACT

Blanching and drying techniques in food processing greatly affect the bioactive values. Research into this effect on soursop leaves raw material in the processing of tea which is a plant with anti-inflammatory, anti-cancer properties. Through a steam blanching process, 4 minutes were selected for compliance with TPC (208.43 ± 0.80 mgTE/g DM) and TFC (43.21 ± 0.71 mgQuercitin/mg DM). Along with the typical antioxidant activities such as TPC, TFC, the TAA, DPPH, ABTS content decreased sharply when heated by convection drying oven at 50-65°C. About 80% of the content is lost at the highest drying temperature. In addition, the Lab* color space of the fresh sample is dark brown transformed by heat, producing a characteristic yellowish hue when extracting custard leaf tea in hot water.

Keywords: Convection drying, total ascorbic acid, total phenolic content, total flavonoid content, antioxidation activity, Soursop (*Annona muricata L.*) leaf tea

INTRODUCTION

Reactions by free radicals in the human body cause significant adverse effects on health **(Formagio et al. 2015)**. Nowadays, studies extract natural compounds are increasingly being promoted, technologies that help to store ingredients with antioxidant activity in processing are of interest **(Pham et al. 2020; Nguyen et al. 2021; Dao et al. 2019)**.
Soursop has a scientific name (*Annona muricata L.)*, a plant native to the Americas and the Caribbean, with the genus Annona of the *Annonaceae* family **(Singh et al. 2017)**. Leaves are green, and glossy **(Nakasone et al. 1998)** with many biological pigments (chlorophyll a, b,…). Natural compounds such as acetogenin are substances found only in the Annonaceae family, so this is a specific group of the importance of this plant family **(Postgraduate et al. 1982)**. The alkaloid annonamine **(Matsushige et al. 2012)**; nornuciferine **(Hasrat et al. 1997)**, sterol,… In addition, it contains carbohydrates (leaf glucose; sucrose, glucose, fructose in fruits; galactomannan in seeds), lipid (semi-dry oil with fatty acids in seeds and leaves), amino acids (prolin, -amino-butyric acid in fruits), polyphenols (caffeic acid, para-coumaric acid, procyanidin, tanins), vitamins (vitamin C in fruits), cyano glycosides **(Moghadamtousi et al. 2015)**.
Some compound polyphenols, such as flavonoid quercetin and flavone (2-phenyl-4H-1- benzopyran-4-one) found in leaves, have a high antioxidant capacity **(Indrawati et al. 2018)**. Leaves of *A. muricata* have a potent antioxidant - a flavonoid found to be more potent apoptosis than clinically established camptothecin anti-cancer agent. These phytochemicals are highly degraded through processing, known to evaporate in some studies to inactivate oxidase enzymes and retain TPC content better than blanching and have limited expense **(Sotome et al. 2009)**. At the same time, the drying process has also been extensively studied on plants and their antioxidant activit. Convection drying technology was chosen because of its ease of implementation, cost savings and high commercial applicability. This study aims to determine the influence of tea processing processes on the content of vitamin C, total phenolic, and antioxidant capacity in soursop leaves.

MATERIAL AND METHODS

Preparations of sample

Soursop leaves were purchased in Tan Phu Dong area, Tien Giang province, Vietnam. Leaves were not pests, torn creased. 1g of sample was extracted with solvent to 50 mL volume. The volumetric flask was placed in a dark space to prepare for analysis.

Determination of total ascorbic acid

The method of titration with 2,6 dichlorophenolindophenol (DCPIP) was previously described by Manas Denre 2014 et al. 2014; Dao et al. 2021 based on dehydroascorbic acid forming reaction and colorless lenco derivative of oxidation of Vitamin C. 10 mL of solution was extracted with distilled water into an erlen flask, add 1 mL of 0.04M HCl. Titration under burettes containing DCPIP to light pink persists for 30s. Record volume and process data.

Determination of total phenolic content

Optical absorbance at 765 nm on UV-Vis spectrophotometer of 0.5 mL of custard leaf sample with 2.5 mL of 10% Folin-Ciocalteu solution, uniform with Votex and let stand for 5 min in the dark with 2.0 mL Na_2CO_3 solution 7.5% left to stand for 1 h was measured. The polyphenol content was expressed in milligrams of gallic acid equivalent in 1g of dry matter (mgGAE /g DM) **(Nhi et al. 2020; Nguyen et al. 2020)**.

Determination of total flavonoid content

The total flavonoids were determined by the colorimetric method described by **Ghasemi et al., 2009; Nguyen et al. 2020** based on the principle that flavonoids form a yellow complex with $AlCl_3$ solution. The color intensity is directly proportional to the flavonoid content determined at 415 nm. Quercetin is used as a reference standard. 0.5 mL of post-extract leaf fluid is added to a test tube with 0.1 mL of 10% $AlCl_3$ reagent, homogeneous for 5 min. Next, add 0.1mL of CH_3COOK 1M solution with 4.3 mL of ethanol. Allow to stand 30 min and measure the level of optical absorption record results.

Determination of free-radicals scavenging activity by DPPH (1,1-diphenyl-2-picrylhydrazyl)

Dilute the sample to the appropriate concentration, 0.5 mL of the diluted sample, into a test tube. The control sample replaces ethanol (99.5%). Then, add a tube of 1.5 mL DPPH solution (OD517 nm = 1.1 ± 0.02) to a test tube and leave it in the dark for 30 min. Measure optical absorbance at 517 nm on a UV-Vis

spectrophotometer. Vitamin C (ascorbic acid) is used as the reference standard **(Nguyen et al. 2020; Yen Nhi et al. 2020)**.

Antioxydation activity by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

Test to assess the ability to prevent the formation of colored oxidized products (734 nm) of ABTS [2,2'-azisbonis (3-ethylbenzothiazolin-6-sulfonic acid) diammonium] of the test substances. The degree of decrease in absorption intensity at 734 nm indicates the antioxidant capacity of the test substance **(Thaipong et al. 2006, Nhi et al. 2020)**.
The scavenging ability of the tea pulp was calculated using the standard equation: $y = 0.126914x + 0.6672952$ **with Regression coefficients R=0.99936.**

Determination of Lab* system

Select collate values L*, a*, b* based on CIE Lab* color space previously described by Torres B et al. 2011. The brightness was measured using a colorimeter Chroma Scanner (NR60CP model). Results were displayed as numbers via L* (brightness ranges from 0-100), values a* (from green to red) and b* (from blue to yellow).

Statistical analysis

Microsoft Excel and Stagraphic software (The Plains corporate, Virginia) were used to support analysis of results based on three replicates. The difference is assessed ANOVA a factor with significance level between means with an error of 0.5%.

RESULTS AND DISCUSSION

Effect of steam blanching on the antioxidant capacity and color of soursop leaves

The content of mg/g dry material of TPC and DPPH affected by blanching time is shown in Figure 1. The results show that blanching time dramatically affects the content of TPC and DPPH. TPC tended to decrease at the heating time of 2 min and 8 min with 159.41 ± 1.55 and 129.76 ± 0.34 mg/g DM, respectively, about 10-26.61% reduction compared to fresh samples. However, in a 4-6 minute heat treatment sample, TPC increased to 17.43 and 9.42%. It can be understood that during heating, 2 min of peroxidase (PE) enzyme treatment is not inactivated, leading to reduced content. In contrast, at 8 min of heating, the longtime lead to the gradual decomposition of a natural compound by heat **(Pinheiro et al. 2018)**. While at 4-6 min provided enough energy to release polyphenols bound to proteins in plant cells, inactivation of PE resulted in increased content extraction. This explanation also correlates with the DPPH content. The concentration after heat treatment with steam at 8 minutes was 35.73 ± 0.72 mg/g DM increased by 15.81 mg compared to fresh samples. In a report from **Zin et al. 2020**, DPPH increased due to blocking the initiation of free radical scavenging reaction chains.

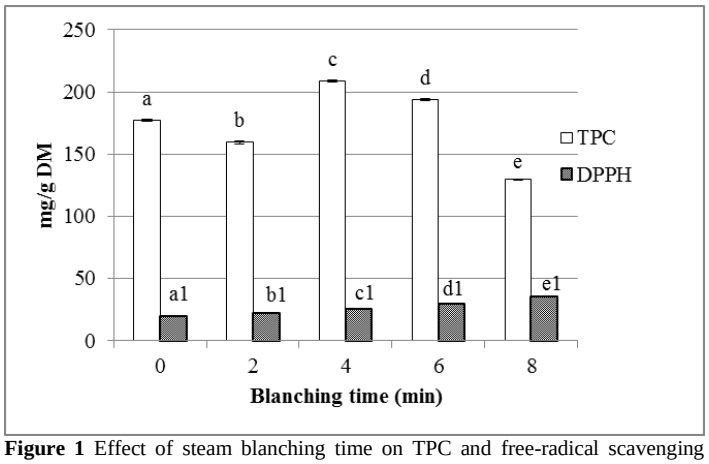

Figure 1 Effect of steam blanching time on TPC and free-radical scavenging activity by DPPH of soursop leaves

The antioxidant activity of soursop leaves was assessed according to the free radical scans ABTS model and the total flavonoid content along with vitamin C is shown in Figure 2. The typical decrease in vitamin C content over 2 min (30.44 ± 0.55 mg/gDM) to 8 min (6.00 ± 0.44) decreased by 19.71%. The sensitivity to heat and light of ascorbic acids was previously reported by **Adefegha et al. 2011**. Through the process of cooking, a large amount of TAA has been identified. Remarkably TFC and ABTS decreased at 2 min of blanching, tended to increase at 4 min and continued to decrease with extended heating time. Significantly increase the content at 4 min 43.21 ± 0.71 and 30.96 ± 0.77. The difference at 2 min and 6 min of TFC is negligible.
Indeed, the increase of TPC, TFC, ABTS during blanching, prominent at 4 min, could be due to a decrease in enzyme-mediated polyphenol degradation (complete inactivation of natural polyphenol oxidase). The increase in total phenolic content may also be due to the release of bound phenolic acids from the breakdown of cellular components of the plant cell walls in leafy vegetables **(Francisco et al. 2010)**. The initial increase in total phenol content in vegetables correlates with that reported by **Liu et al. 2002** who reported that chilling or blanching can increase the phenol content in vegetables. TPC, TFC, ABTS Values are reported to be closely related. **Hongyi Sun et al. 2018** present the relationship of these values in Okara, between TPC and TFC with R^2 of 0.920, TPC and ABTS of 0.960, so the increase of TPC also leads to the same trend in TFC and ABTS values. Furthermore, the differences in TPC, TFC, ABTS, DPPH, and Vitamin C may be due to differences in the plant matrix, the amount of solvent that can attract compounds capable of scavenging free radicals, and compounds are easily modified by conditions such as temperature, light **(Sun et al. 2018)**.

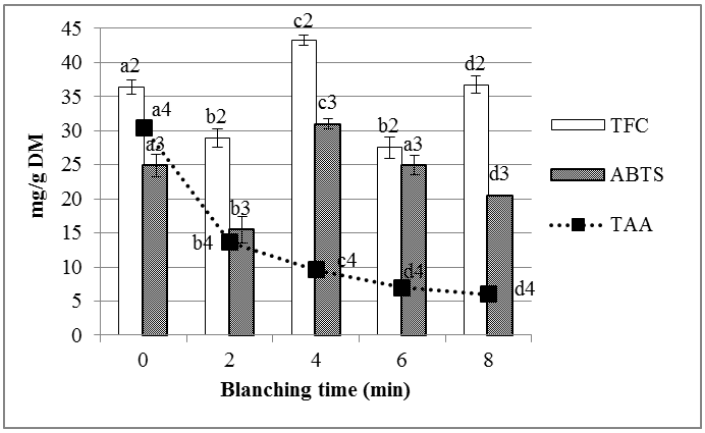

Figure 2 Effect of steam blanching time on TFC, ABTS and TAA of soursop leaves (a-d means different in the analytical samples; 1 for TFC, 2 for ABTS, 3 for TAA)

Reports on the effect of blanching parameters on leaf color are shown in Table 1. The difference was negligible in the L* and b* values of blanched and control samples and TDC values between the samples after blanching. a* values at 4 minutes of blanching differ significantly from fresh samples indicating a decrease in the green of the sample. At the same time, there is a difference in the color system after blanching. This may explain the color pigments like chlorophyll in the leaves are separated by heat to make the color darker than fresh samples **(Quenzer et al. 1977)**. Plant pigments (chlorophyll, carotene) are heat-treated decay that can turn into products with antioxidant activity **(García-Herrera et al. 2013; Pinheiro et al. 2018)**. **Kessy et al. 2016** points to the release of flavonoid compounds in blanching on lychee and green vegetables. Several studies have shown that, under the effect of steam blanching, the phytochemical compound in food is governed by cooking method, temperature and portion size **(Hwang et al. 2012)**.

Table 1 The effect of blanching time on CIE Lab's color system and the total different color (TDC) of soursop leaves ($^{a-e}$ significant difference between rows)

Blanching sample	Fresh	2 min	4 min	6 min	8 min
L* (^{a})	33.33 ± 1.53	31.07 ± 1.53	31.21 ± 2.47	33.23 ± 3.09	32.21 ± 0.82
a*	-10.15 ± 0.05^{a}	-9.37 ± 0.46^{abc}	-8.34 ± 1.10^{de}	-9.87 ± 1.87^{ab}	-7.88 ± 0.02^{b}
b* (^{a})	11.59 ± 0.63	9.52 ± 0.83	9.06 ± 2.17	11.90 ± 2.54	10.56 ± 0.43
TCD (^{a})	0	3.52 ± 2.82	4.40 ± 2.58	4.53 ± 0.95	2.87 ± 0.79

Effect of convection drying temperature on the antioxidant capacity and color of soursop leaves

From the survey studies of Figures 1 and 2, the blanching process parameter was selected for soursop leaves for 4 minutes. The drying process was finished after the leaf moisture content was less than 10%. The results of the antioxidant content retention through blanching and drying processes are shown in Figure 3. In particular, phytochemical compounds are significantly reduced through heating. The total phenolic content decreased by 30.92% after ANOVA treatment with a significance level of $\alpha = 0.05$ through blanching and continued to decline sharply after drying at 50°C (31.69), reducing by 68.31%. No significant difference in TPC and ABTS between samples 55 and 60 °C. The lowest retention content after drying at 65°C is 12.70% (TPC), 43.36% (TFC), 18.60% (DPPH), and 17.12% (ABTS). The result is consistent with a few studies when making comments that, after processing, the ability to catch free radicals in soursop leaves decreased **(Powers et al. 2004)**. Although some researchers stated that there is a degradation in the content of antioxidant compounds through the drying process, temperature and drying time were correlated with this reduction **(Farhana et al. 2018; Balois-morales et al. 2019)**. The increase or decrease in content after blanching depends on the manipulation, extraction conditions and the season of each sample of soursop leaves. On the other hand, analyzing the total process is affected by the sample weight in one process.

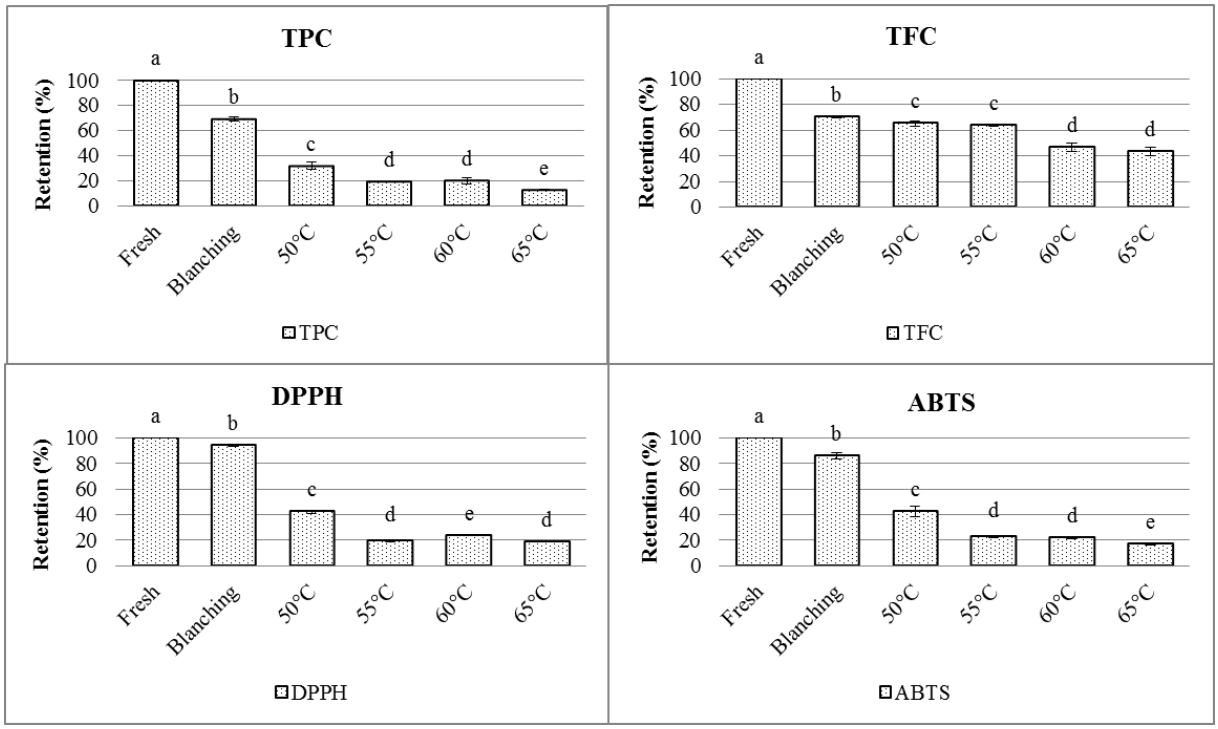

Figure 3 Retention TPC, TFC, DPPH, ABTS after blanching and convection drying process (50-55-60-65°C)

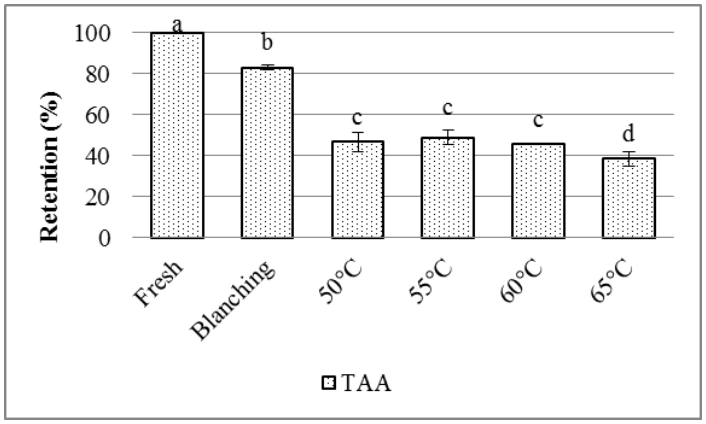

Figure 4 Effects of blanching and drying process on retention of ascorbic acid

Figure 4 shows a decrease in ascorbic acid content in plant samples when heat treated at 4 minutes of blanching and drying temperatures from 50-65°C. Of which, 16.96% ± 1.26 TAA losses due to steam heat, continue to decrease by about 47% retention in 50-55-60°C. There is no significant difference at these three temperatures. The lowest at 65°C, exists at 38.66% ± 3.51. Ascorbic acid is a compound sensitive to light, temperature and, air. After heating, the vitamin C molecule decays and is easily soluble in water, capable of being influenced by the listed factors and attracted by the drying agent out of the drying material. Previously, reports of TAA losses in plant materials were published **(Zheng and Lu 2011; Oboh et al. 2005; Taylor et al. 2010)**.

The color change follows the increasing trend of L* value from 33.33 ± 1.53ab to 42.41 ± 4.31b at 65 ° C. However, the color angles a* and b* indicate that the yellow and brown fall after heating. In addition, the total different color increased significantly with increasing drying temperature. The highest difference at 65 °C was 14.25 ± 2.25d compared to fresh samples. This determines that the color is affected considerably after drying, the high temperature destroys the specified color shades, and the Maillard reaction causes darkening when heated for a long time. This has also been previously reported for cabbage **(Powers et al. 2004; Alibas 2008; Abu-ghannam 2015)**.

Table 2 CIE Lab* system of soursop leaves through blanching and drying process ($^{a-e}$ significant difference between rows)

Sample	Fresh	Blanching	50°C	55°C	60°C	65°C
L*	33.33 ± 1.53^{ab}	31.21 ± 2.47^{a}	40.30 ± 4.00^{cd}	39.16 ± 2.54^{cd}	41.31 ± 7.89^{b}	42.41 ± 4.31^{b}
a*	-10.15 ± 0.05^{a}	-8.34 ± 1.10^{b}	-3.32 ± 0.20c	-5.36 ± 0.39^{d}	-2.67 ± 1.51^{c}	-3.55 ± 0.59^{c}
b*	11.59 ± 0.63^{ab}	9.06 ± 2.17^{a}	13.86 ± 0.97^{cd}	14.67 ± 1.88^{b}	13.74 ± 1.85^{cd}	14.48 ± 1.90^{cd}
TCD	0	4.40 ± 2.58^{a}	11.92 ± 2.28^{bc}	9.53 ± 2.46^{b}	13.50 ± 2.37^{bc}	14.25 ± 2.25^{d}

CONCLUSION

The retention of color values and the content of antioxidants has been significantly reduced after blanching and convection drying. For small sample volumes, blanching at 4 minutes resulted in a higher TPC and TFC content than fresh samples because of the inactivation of oxidase enzymes. However, during the same simultaneous drying process, this content was reduced by a large total sample mass, and decreased after drying, with losses of more than 50% for TAA and 80% for chemicals that differs by hot air stream agent for a long time. The color varies slightly in low a* and TDC values between blanching and high drying samples. The limitation of the study is the need for an assessment of the effect of drying time on the quality of soursop tea leaves and the content of antioxidants after extracting tea leaves with hot water of users.

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