17798-49593-1-RV.docx

The Impact of Vitamin C Addition to Stability of Antioxidant Activity in Green Tea

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ABSTRACT

Green tea is a popular drink for Indonesians. One of the ingredients in green tea is catechins. Catechins, especially the EGCG type, have very high antioxidant power, so many studies have used catechin active substances, especially EGCG, as anticancer substances, preventing heart disease, diabetes, and obesity. One of the disadvantages of catechins is that they are very unstable, especially in the form of a solution or brew. According to several studies, vitamin C can stabilize the catechin content. So this study focuses on the impact of vitamin C addition on the antioxidant activity of green tea brew. **Objective:** To obtain the percent increase in IC50 before and after adding vitamin C to green tea brew during the 4-day storage time. **Methods:** Antioxidant activity (IC50) testing was conducted on six groups of samples, namely Green Tea (TH), Green Tea + Vitamin C 1 mg (THVC1), Green Tea + Vitamin C 1.5 mg (THVC2), Green tea + Vitamin C 2 mg (THVC3), Green Tea + Vitamin C 2.5 mg (THVC4) and Green Tea + Vitamin C 3 mg (THVC5). Each group of samples was tested for antioxidant activity after 0 days, 1 day, 2 days, 3 days, and 4 days of storage. Each storage time was tested for antioxidant activity in three replicates. Storage conditions were carried out at 4oC with storage in a dark and tightly closed bottle. **Conclusion:** Vitamin addition has a significant effect on preventing the increase in IC50 in green tea brew. The percent increase of IC50 during 4 days of storage time in TH, THVC1, THVC2, THVC3, THVC4, and THVC5 was 227%, 125.50%, 111.73%, 79%, 67.29%, and 66.52%, respectively

***Keywords**: Green Tea, Antioxidant activity, Vitamin C*

Introduction

Tea is a very popular beverage around the world. Tea is also one of Indonesia's main agricultural products and export commodities. Indonesia ranks third in consuming green tea, with about 30,000 metric tonnes annually.

Green tea has an antioxidant power of 13.51 μg/ml in a DPPH concentration of 20 mg/ml (Pereira et al., 2014). Green tea contains various compounds such as polyphenols, organic acids, amino acids, methylxanthines, carbohydrates, minerals, volatile compounds, and vitamins. Polyphenols are the largest content compared to other contents. Polyphenols consist of 30-40% catechins, 5-10% flavonols, and ester groups (depside) of 2-4%. Catechins are a type of antioxidant in green tea which consists of several types such as EC, ECG, EGC, and EGCG (Rady et al., 2018).

In several studies, adding vitamin C or ascorbic acid can stabilize the catechin content. Vitamin C has several elements that can regenerate degraded EGCG. The structure of ascorbic acid consists of a lactone and two enolic hydroxyls, as well as a primary alcohol and a secondary alcohol. The enolic hydroxyl (enediol) is an important part of the ascorbic acid structure as an antioxidant. Enediol can be oxidized easily to form diketones (Jeanmonod et al., 2018). Vitamin C and catechins will experience synergistic effects. The synergistic effect occurs due to differences in the reduction potential of various antioxidants from the same system. The reduction potential of EGCG is 430 mV, and vitamin C is 282 mV. The synergistic effect occurs when H+ ions released from vitamin C will regenerate radical EGCG into non-radical EGCG (Dai et al., 2008). So that EGCG is not degraded to form dimer compounds and theasisnensin A. Vitamin C, which releases H+ because it is oxidized, will then form into oxalic and threonic acids (Truffault et al., 2017).

Some studies mentioned that adding ascorbic acid to EGCG can increase EGCG's ability to inhibit lung cancer cells and protect EGCG from oxidation (Li et al., 2010). In another study, Scalia et al. in 2013 stated that the % photodegradation of EGCG was greater than the % photodegradation of EGCG with the addition of ascorbic acid. Therefore, in this study, vitamin C was used with variations in the addition of levels to increase the stability of antioxidant activity in green tea brew. The levels of vitamin C added in this study are 1 mg, 1.5 mg, 2 mg, 2.5 mg, and 3 mg. So it is expected that the temperature conditions, brewing extraction methods, storage containers, and the addition of vitamin C carried out in this study will stabilize EGCG levels and antioxidant activity in green tea brew.

The antioxidant activity test method was carried out on green tea brew, vitamin C, and added vitamin C. Several ways could be used to test the antioxidant activity of green tea. Several ways can be used to test antioxidant activity, namely DPPH (Alam & Bristi., 2013), FRAP or Ferric Reducing Antioxidant Power (Rock & Brunswick., 2005), TPC or Total Phenolic Content, and DPPH-HPLC (Kumara et al., 2018). The DPPH method was chosen in this study because it is more efficient and in accordance with the analyzed analyte.

Based on the explanation above, the research will be conducted on antioxidant stability efforts by adding variations in vitamin C levels of 1 mg, 1.5 mg, 2 mg, 2.5 mg, and 3 mg to green tea brew using ultrasonic extraction brewing method, storage at 4oC and using dark and tightly closed bottles as storage containers. DPPH method with UV-Vis spectrophotometer measurement was chosen for antioxidant activity analysis. Antioxidant activity was measured at 0 days, 1 day, 2 days, 3 days, and 4 days of storage. By knowing the antioxidant activity of green tea samples before and after adding vitamin C, the % increase in antioxidant activity of green tea brew during storage will be known.

Research methods

1. Research sample

The sample used in this study was green tea powder with a local brand from Surabaya-Indonesia.

2. Research Materials and Tools

The materials used are green tea powder, Vitamin C from Sigma Chemical, methanol pro analysis (full-time), and DPPH (Sigma Aldrich).

The tools used in this study are test tubes, volume pipettes 1 mL, 2 mL, 3 mL, 4 mL, 5mL and 6 mL, drop pipettes, measuring cups 5 mL, measuring flasks 5 mL, 10 mL, 25 mL, and 100 mL, analytical scales, micro-analytical scales, ultrasonic shaking water bath, chamber, and UV-Vis Spectrophotometry.

3. Methods

Analysis of Antioxidant Activity in Green tea, Vitamin C addition and vitamin C use DPPH method and spectrofotometri UV-Vis

Results and Discussion

1. Determination of Antioxidant Activity

Determination of antioxidant activity begins with determining the stability of DPPH first. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a very stable free radical molecule, so it was used in this study to measure the antioxidant activity of green tea brewed without the addition of vitamin C and green tea that has been added vitamin C in various levels during a certain storage time. DPPH is used in the form of black powder. Then the DPPH powder was dissolved in methanol p.a for analysis. DPPH requires a certain time to react completely with antioxidant samples. Knowing the optimal time required for DPPH to react completely will prevent measurement errors, which will result in invalid data obtained. So it is necessary to test the stability of DPPH.

DPPH stability is tested by measuring the absorbance stability of DPPH mixed with the sample at a wavelength of 515 nm using a UV-Vis spectrophotometer. It was found that the absorbance of DPPH began to stabilize at the 55th minute until the 70th minute. So the time required for DPPH to react completely with the antioxidant sample is about 55-70 minutes.

2. Antioxidant Activity of Vitamin C

The antioxidant activity of vitamin C needs to be measured to compare with the antioxidant activity of green tea brew and the mixture of green tea brew and vitamin C. The antioxidant activity of vitamin C was tested using DPPH. Vitamin C was prepared in series (500 ppm, 300 ppm, 200 ppm, 100 ppm, 70 ppm, 50 ppm, 30 ppm, and 10 ppm). Then each level series was reacted with DPPH. After reacting completely, the absorbance of DPPH was measured at a wavelength of 515 nm, and then the % inhibition of each level was measured. A logarithmic curve will be obtained based on the concentration data/log concentration vs % silencing data. So that IC50, or the level of vitamin C needed to reduce the oxidation of DPPH 50%, is 120.226 ppm.

3. Antioxidant Activity of Brewed Green Tea

Table 1 : IC_{50} TH,THVC1,THVC2,THVC3,THVC4 and THVC5

Storage time	IC_{50} (ppm)					
	TH	THVC1	THVC2	THVC3	THVC4	THVC5
0 day	2342,30 ± 794,85	2283,28 ± 483,42	2181,96 ± 152,45	2285,01 ± 407,40	2263,32 ± 245,91	2174,10 ± 1455,43
1 day	3233,31 ± 424,89	2679,79 ± 559,19	2650,19 ± 265,21	2562,95 ± 105,69	2151,223 ± 445,30	2136,75 ± 64,17
2 day	3251,50 ± 139,31	2709,75 ± 21,19	2437,48 ± 225,71	2385,57 ± 555,17	2320,79 ± 232,21	2183,86 ± 97,94
3 day	3417,47 ± 392,86	2789,89 ± 99,18	2474,50 ± 53,45	2284,96 ± 63,88	2264,05 ± 710,94	2197,22 ± 50,55
4 day	7663,21 ± 138,47	5141,87 ± 112,78	4619,97 ± 76,45	4106,97 ± 37,16	3786,40 ± 240,61	3620,41± 85,69
$t_{1/2}$	2,33 day	3,41 day	3,69 day	4,73 day	5,39 day	5,44 day

*$t_{1/2}$: half-life

Antioxidant activity was measured on 6 samples, namely TH, THVC1, THVC2, THVC3, THVC4, and THVC5 extracts. The IC50 value of each sample indicates the antioxidant activity at storage time from day 0 to day 4. The IC50 value data on the samples can be seen in Table 1.

Based on the data in Table 1, the addition of vitamin C will decrease the IC50 value, and the more vitamin C added, the IC50 value will decrease, and the longer the storage time, the greater the IC50 value of the sample. It shows that green tea brewed without the addition of vitamin C and with the addition of vitamin C stored for a longer time will reduce antioxidant activity. Each sample has the lowest IC50 value on day 0, so the green tea brew without the addition of vitamin C and with the addition of vitamin C is recommended to be consumed immediately after manufacture.

4. Impact of Vitamin C on the Stability of Antioxidant Activity of Brewed Green Tea

Based on Table 2, the percent increase in IC50 during the 4-day storage time of TH, THVC1, THVC2, THVC3, THVC4, and THVC5 extracts were 227%, 125.50%, 111.73%, 79%, 67.29%, and 66.52%, respectively. The IC50 value in each sample has a significant difference based on a statistical calculation of one-way ANOVA ($p<0.05$). This value indicates that adding vitamin C affects the IC50 of green tea brew during storage.

Table 2 Percent Increase in antioxidant activity (IC50) at each storage time

Storage Day	% Kenaikan IC_{50}					
	TH	THVC1	THVC2	THVC3	THVC4	THVC5
1	38,04%	17,37%	21,46%	12,16%	(4,95%)*	(1,72%)*
2	38,82%	18,678%	11,71%	4,40%	2,54%	0,45%
3	45,90%	22,19%	13,41%	*	0,032%	1,06%
4	227%	125,20%	111,73%	79%	67,29%	66,52%

Conclusion

The addition of Vitamin C affects the stability of antioxidant activity. It is evidenced by the percent increase in IC50 during the 4-day storage time of TH, THVC1, THVC2, THVC3, THVC4, and THVC5 samples which are 227%, 125.50%, 111.73%, 79%, 67.29%, and 66.52% respectively. These data concluded that green tea brewed without the addition of vitamin C had a very high % increase (small antioxidant stability) compared to green tea brewed with vitamin C (large antioxidant stability).

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