RESEARCH ARTICLE

A study of the stability of antioxidant activity of green tea extract powder with vitamin C

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Abstract

Background: Green tea contains Epigallocatechin gallate (EGCG), the highest antioxidant activity. EGCG is an unstable compound due to degradation. Vitamin C is thought to be able to regenerate EGCG radicals into normal forms and increase green tea's antioxidant activity. **Objective:** The aim of this research was to determine the effect of adding vitamin C in maintaining the stability of the antioxidant activity of green tea. **Method:** This research was carried out by using UV-Vis spectrophotometry to determine the rate of decreasing antioxidant activity at 0 hours, six hours, and 24 hours by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. **Result:** IC_{50} values of green tea before and after the addition of vitamin C were obtained at 147.53 ppm and 97.75 ppm, respectively. The degradation rate of green tea before and after the addition of vitamin C was 0.4228 %/hour and 0.3231 %/hour, respectively. **Conclusion:** The antioxidant activity of green tea with vitamin C is greater than without vitamin C addition. The degradation rate of green tea with vitamin C is smaller than green tea only, so the authors suggest the addition of vitamin C to green tea extract powder products to increase the stability of its antioxidant activity.

Introduction

Tea (*Camellia sinensis*) is the most consumed beverage in the world after water which is about two-thirds of the population (Khan and Mukhtar, 2013). In Indonesia, tea is one of the plantation commodities that has an important role in economic activities and one of Indonesia's export commodities (Badan Pusat Statistik, 2019). The most abundant content in tea is polyphenols, one of which is catechins. Catechins consist of several types such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and Epigallocatechin gallate (EGCG). Green tea contains EGCG amounting to 59% of the total catechins, which contribute greatly in antioxidant activity (Rady *et al.*, 2018). Antioxidants are molecules that can inhibit free radicals so as to block the chain response that can cause oxidative stress (Tsao, 2015).

EGCG is an unstable compound because it can be degraded to form other compounds. if auto-oxidation occurs, EGCG changes to Theasinensin A form, but if epimerisation occurs, GCG compounds will be obtained (Sang *et al.*, 2011). Therefore, EGCG in green

tea must maintain stability to reduce degradation. This research studied the effect of adding vitamin C as antioxidant to maintain the stability of EGCG. Vitamin C and EGCG give a synergistic effect that occurs due to the difference in the reduction potential in the same system. EGCG has a reduction potential of 430 mV and vitamin C of 282 mV (Tsao, 2015). Vitamin C and EGCG give a synergistic effect that occurs due to the difference in the reduction potential in the same system. EGCG has a reduction potential of 430 mV and vitamin C of 282 mV (Tsao, 2015). The synergistic effect occurs because H^+ ions released from vitamin C will regenerate EGCG radicals into normal forms of EGCG (Dai *et al.*, 2008).

In this study, the effect of the addition of vitamin C will be observed on the stability of the antioxidant activity of green tea extract during a certain storage time. Brewing green tea extract is carried out at a temperature of 70°C for 20 minutes. Aluminium foil-covered test tubes are used as storage so that they are not exposed to light and stored at room temperature (25°C) (Zeng *et al.*, 2017).

The method used to determine the antioxidant activity is DPPH (2,2-diphenyl-1-picrylhydrazyl) method using UV-Vis spectrophotometer. DPPH is a stable free radical. The measuring principle of the DPPH method is the colour change from purple to yellow when reacting with antioxidants (Kedare and Singh, 2011).

Methods

Green tea extract solution preparation

Concentration of 1000 ppm of green tea extract solution was freshly prepared from 50 mg of green tea extract in 50 mL of aqueous solution at 70˚C. Five concentrations of solution were made at the level of 40, 80, 120, 160, 200 ppm.

Vitamin C solution preparation

Concentration of 1000 ppm of vitamin C solution was freshly prepared from 50 mg of vitamin C standard in 50 mL of aqueous solution. Five concentrations of solution were made at the level of 12, 16, 20, 30, 40 ppm.

EGCG solution preparation

Concentration of 1000 ppm of EGCG solution was freshly prepared from 50 mg of EGCG standard in 50 mL of aqueous solution. Five concentrations of solution were made at the level of 2, 4, 6, 8, 10 ppm.

Vitamin C addition on green tea extract

A concentration of 0.25 mL of green tea extracts 80, 160, 240, 320, and 400 ppm were taken with a micropipette, placed in a test tube covered with aluminium foil and to each was added 0.25 mL of vitamin C 80 ppm.

DPPH solution preparation

Concentration of 50 ppm of DPPH solution was freshly prepared from 5 mg of standard DPPH in 100 mL of ethanol solution. Then the concentration of DPPH 25 ppm in ethanol solution was made.

Maximum wavelength optimisation of DPPH

The maximum wavelength of DPPH was measured in DPPH solutions (50 and 25 ppm). The wavelength which showed the highest absorbance was chosen for the next analysis. The test was performed using a UV-Vis spectrophotometer at a wavelength of 400-800 nm.

Validation

Specificity

The specificity test is used to determine if the method used can specifically measure the desired analyte (Yuwono and Indrayanto, 2005). It was also used to prove that the absorbance of DPPH was not disturbed by the absorbance of the samples, namely green tea extract, vitamin C, and EGCG. A volume of 0.5 mL of each sample was taken and added to 2.5 mL DPPH 25 ppm. The test was performed using a UV-Vis spectrophotometer at a wavelength of 400-800 nm. Sample analysis was also carried out without the addition of DPPH.

Linearity

The linearity test was carried out by preparing five different concentration solutions of green tea extract, vitamin C, and EGCG. A volume of 0.5 mL of each sample was taken and added to 2.5 mL DPPH 25 ppm. All the samples' solutions were scanned using a UV-Vis spectrophotometer. A regression line was made from the concentration of samples (x) and absorbance (y).

Accuracy

The accuracy test was analysed by the addition of three different concentrations (80%, 100%, and 120%) to the samples. Three replicates were made for each concentration of EGCG standard addition. All of the samples, EGCG standard, and sample solution without standard addition were scanned using UV-Vis spectrophotometry. The accuracy requirement was assessed based on the recovery. The recovery for 1 - 10 ppm of EGCG in the sample was between 80-110% (Yuwono and Indrayanto, 2005).

Precision

The precision test was analysed on a 100% concentration green tea extract solution (six replicates). A volume of 0.5 mL of each sample was taken and added to 2.5 mL DPPH 25 ppm. Then, all samples were scanned using UV-Vis spectrophotometry. Precision requirements were assessed from the value of RSD that ≤ 2% (Yuwono and Indrayanto, 2005).

Antioxidant activity test

Antioxidant activity tests were performed on samples of green tea extract, vitamin C, and green tea extract with the addition of vitamin C. A volume of 0.5 mL of each sample (five concentrations) was taken and added to 2.5 mL DPPH 25 ppm, with the exemption of green tea extract with the addition of vitamin C which

was directly reacted with 2.5 mL DPPH 25 ppm. Afterwards, the mixture was vortexed, incubated for 30 minutes and scanned using a UV-Vis spectrophotometer. The solution was kept for six and 24 hours and repeated.

Results

Wavelength optimisation

The maximum wavelength in 50 ppm and 25 ppm of DPPH standard solution was 517 nm. This maximum wavelength was used in a UV-Vis spectrophotometry analysis.

Validation

Specificity

After overlaying the spectra, the measured absorbance is true DPPH and DPPH absorbance is not disturbed by the absorbance of green tea extract, vitamin C, and EGCG, so the method used is specific to detect DPPH.

Linearity

Linearity tests were performed on green tea extract, vitamin C, and EGCG. Green tea had a concentration range of 39.80 ppm - 198.00 ppm. Vitamin C, 11.88 ppm-39.60 ppm and EGCG, 2.01 ppm-10.06 ppm. Linearity evaluation using correlation coefficient parameter (R) was close to one and the value of relative process standard deviation value (Vxo) was

not more than 5%. On all samples, the linearity test meets these requirements.

Accuracy

Acceptance criteria of recovery for analyte concentrations of 1-10 ppm are 80-110%. The recovery of 80%, 100%, and 120% of EGCG standard addition were 98.05% ± 0.05, 92.38% ± 0.05, and 98.41% ± 0.02, respectively. The result indicated that this method was accurate.

Precision

Acceptance criteria of RSD for precision is ≤ 2%. The result of RSD was 0.71% ± 0.30. The results suggested that this method was precise.

Antioxidant activity

After testing using UV-Vis spectrophotometer, the calculation of percent inhibition and IC_{50} of each sample was done. The stability is carried out by calculating the rate of degradation of antioxidant activity (%/hour). The results are in Figure 1. Percent inhibition was calculated by using the formula below:

$$\% \text{ inhibition} = \frac{A-B}{A} x 100\%$$

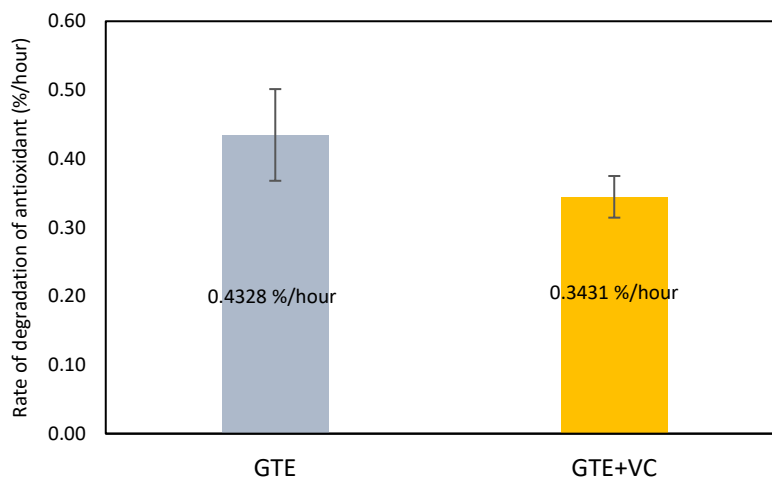A = absorbance of DPPH
B = absorbance of residual DPPH

Figure 1: The rate of degradation of green tea extract (GTE) decreased after receiving additional vitamin C

Discussion

All samples showed that with increasing concentration there was an increase in percent inhibition so that the ability to inhibit free radicals was also greater, but in vitamin C stored 24 hours only a concentration of 39.60 that can provide antioxidant activity. This is because the concentration of vitamin C can affect its stability, where the greater the concentration, the lower the rate of degradation (Yin *et al.*, 2022). In solution, vitamin C is so easily oxidized that in just 24 hours almost all concentrations of vitamin C is degraded leading to loss of ability to inhibit free radicals (Dewaantari *et al.*, 2021). Therefore, the combination of vitamin C and EGCG in solution cannot endure for a day, and it is advisable to make the mixture in dry form.

IC_{50} value of green tea extract, and green tea extract with the addition of vitamin C were 147.53, and 97.75 ppm, respectively. All samples showed that the IC_{50} value increased, and antioxidant activity decreased on longer storage. This is due to the degradation of the polyphenol structure in both green tea extract and vitamin C. The degradation of EGCG contained in green tea extract occurs due to the oxidation of the polyphenol structure. EGCG has three hydroxyl groups on the B-ring and a gallate group on the C-ring, which have antioxidant activity. However, this EGCG structure is susceptible to autoxidative degradation.

EGCG autooxidation results in the formation of hydrogen peroxide as well as the production of products such as Theasinensin-A which can decrease its efficacy (Furniturewalla and Barve, 2022). On the other hand, it is possible that EGCG epimerises into GCG due to the preparation of a solution of green tea extract at a temperature of 70˚C (Krupkova *et al.*, 2016).

The addition of vitamin C can reduce IC_{50} value in green tea extract. Vitamin C which is added to green tea extract will interacts synergistically with EGCG in the green tea extract and produce a lower IC_{50} value. This condition occurs due to the large reduction of potential difference between vitamin C and EGCG (Tsao, 2015). In addition, vitamin C has a small IC_{50} value so that it has great antioxidant activity, when added to green tea extract it will help increase antioxidant activity of green tea. Based on Figure 1, the average slope in green tea extract with the addition of vitamin C is smaller than the green tea extract alone, which is 0.3231 %/hour and 0.4228 %/hour (sig = 0.049). Smaller slope value indicates that the rate of degradation in antioxidant activity is smaller so it can be said to be more stable.

To stabilize EGCG, vitamin C acts as a reducing agent by releasing electrons so that it can change from the EGCG radical to normal as shown in Figure 2 (Dai *et al.*, 2008).
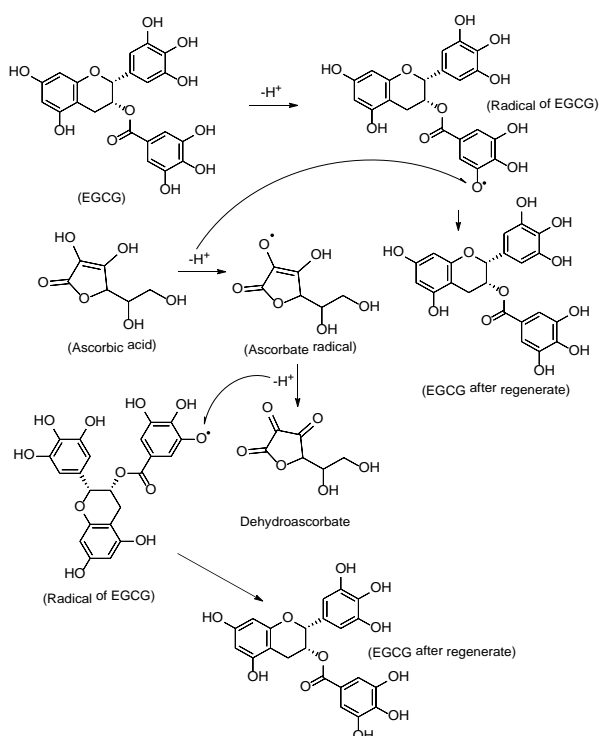

Figure 2: Prediction of EGCG stability reaction by vitamin C (Rahman *et al.*, 2019)

Conclusion

The antioxidant activity of green tea with vitamin C is greater than without vitamin C addition. The degradation rate of green tea with vitamin C is smaller than green tea only. On the other hand, the addition of vitamin C can also improve the stability of the antioxidant activity of green tea extract.

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