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Antioxidant activity of Butterfly Pea Flower Kombucha (*Clitoria ternatea*)

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ABSTRACT

Kombucha is a traditional fermented beverage from sugar and tea by SCOBY, a kombucha starter culture. Kombucha can be derived from various substrates such as black tea, green tea, oolong tea, and herbal tea. The butterfly pea flower (*Clitoria ternatea*) is a popular herbal tea ingredient known for its vibrant blue color and potential health benefits. In this study, butterfly pea flowers were utilized as the substrate for brewing kombucha. This study aims to determine butterfly pea flower kombucha's antioxidant activity and vitamin C content. IC_{50} antioxidant activity of infusion butterfly pea flower was 195,4 ppm. After 12 days of fermentation, the IC_{50} antioxidant activity of butterfly pea kombucha was 233,3 ppm. Throughout the fermentation process, there was a continuous decline in vitamin C levels, starting at an initial concentration of 191,6 ppm and reaching 74,0 ppm at the end. During kombucha fermentation, antioxidant activity increases while vitamin C levels decrease.

Introduction

Antioxidants play a crucial role in inhibiting the oxidation process. They are vital for the body as they help prevent oxidative stress associated with various degenerative diseases. Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body[1]. In cases where the body lacks its own (endogenous) antioxidants, external sources of antioxidants become necessary[2]. Natural antioxidants are abundant in various food items,

such as tea, spices, chocolate, leaves, vegetables, seeds, and protein- and enzyme-rich ingredients[3]. Kombucha is one such source that can provide these beneficial antioxidants.

Kombucha is produced through traditional fermentation by fermenting a solution comprising tea and sugar, using a starter culture of kombucha known as SCOBY (Symbiotic Culture of Bacteria and Yeast). This beverage exhibits distinctive sensory attributes, including a sweet-sour taste and a unique aroma. It is enriched with essential minerals, vitamins, and organic acids[4]. Kombucha can be derived from various ingredients, such as green tea, black tea, bay leaves, and Dutch teak leaves. One of these ingredients, for instance, can be sourced from butterfly pea flowers.

The butterfly pea (*Clitoria ternatea*) is a flower with a distinct purple petal. Butterfly pea flower is one of the plants as a source of antioxidants. The ingredients in *Clitoria ternatea* flower extract are several bioactive compounds whose role is as natural antioxidants and flavonoid compounds are one of them[5].

Vitamin C, known for its powerful antioxidant properties, acts as a strong reducing agent and effectively scavenges free radicals[6]. It plays a crucial role in the initial line of defense against oxidative stress. According to Abaci *et al*, the components responsible for the antioxidant properties of kombucha include polyphenols, water-soluble vitamins such as B1, B2, B6, B12, and C, as well as various organic acids like acetic, gluconic, glucuronic, lactic, tartaric, citric, and malic acids[7]. This study aimed to determine the activity of antioxidant and vitamin C content from butterfly pea flower kombucha.

Method

1. Preparation of kombucha

According to Ahmed *et al*, making kombucha with modification. As much as 1 gram of dried butterfly pea flowers is brewed using boiling water as much as 200 ml for 15 minutes and then drained into a sterile glass container/jar[16]. Refined sugar is put into the container as much as 20 grams. The container is closed and waited until the temperature drops to around 30°C. The starter is added as much as 10% (12 ml of liquid starter & 8 grams of SCOBY starter). The jar is covered with sterile gauze and then incubated for 12 days at room temperature (±28-30°C.

2. Determination of antioxidant activity

Determination of the antioxidant activity of butterfly pea flower kombucha, according to Mubarak's research namely testing, is carried out in several stages. The first is the preparation of mother liquor[8]. A total of 7.9 mg of DPPH was weighed and dissolved in 50 ml of methanol Pa to obtain a concentration of 0.4 mM. The second is the preparation of the control solution. The mother liquor with a concentration of 0.4 mM was homogenized and then incubated for 30 minutes at 37˚C. Third, the maximum wavelength of DPPH is determined by measuring its absorbance at a wavelength of 515 nm. The fourth is testing the antioxidant activity using the DPPH method. Antioxidant activity was tested by adding 1 ml of DPPH solution (0.4 mM) to 50 µl of sample solution and then adding 5 ml of methanol pa. Incubated for 30 minutes at 37˚C and measured the absorbance at a wavelength of 515 nm. The antioxidant activity of the sample is determined by the magnitude of the resistance to DPPH radical uptake by calculating the percentage inhibition of DPPH uptake by using the formula: $\%Inhibisi$ = (Abs. blank-Abs. sample)/(Abs. blank) = 100%, where Abs Blank is the absorption of DPPH radicals in wavelength of 515 nm, and Abs Sample is the absorption of the sample in DPPH radicals at a wavelength of 515 nm. The amount of antioxidant activity is determined by using the IC_{50} value. The IC_{50} value is the antioxidants needed to minimize 50% DPPH radicals. The IC_{50} value is obtained from the linear regression formula, which states the correlation between extract levels on the x-axis and % radical capture on the y-axis.

3. Determination of vitamin C content

The analysis of vitamin C content was performed using the iodometric titration method. Firstly, 25 ml of kombucha tea was measured using a dropper pipette and recorded as the initial weight. It was then diluted with 100 ml of distilled water. Next, 10 ml of the sample was transferred into a 250 ml Erlenmeyer flask. Subsequently, 2 ml of 1% amylase indicator solution was added, and the mixture was titrated with 0.01 N iodine solution until it turned blue. The usage of 0.01 N iodine solution was as follows: 1 ml of iodine solution = 0.88 mg of ascorbic acid (Vitamin C). The calculation for the vitamin C content is as follows: Vitamin C mg/100 ml = (A × FP × 0.88)/W, where A (volume of iodine solution used for titration), FP (dilution factor) and W (weight of the sample).

4. Data analysis

The data analysis was conducted descriptively. The antioxidant activity of the sample was measured by determining the inhibition of DPPH radical absorption. This can be calculated as the percentage of inhibition. The antioxidant activity was determined by IC_{50} (Inhibition Concentration 50%), which represents the concentration that inhibits free radicals by 50%. IC_{50} is determined based on the percentage of inhibition data, and a linear regression equation is obtained to express the relationship between the concentration of the extract on the x-axis and the percentage of radical scavenging on the y-axis.

Results and Discussion

In this particular research, a 12-day fermented kombucha infused with butterfly pea flowers was utilized. The effectiveness of the compound as an antioxidant was determined using the IC_{50} parameter. IC_{50} represents the concentration of antioxidants required to reduce 50% of DPPH radicals. A decreased IC_{50} value indicates that the extract is more effective in scavenging DPPH radicals or functioning as an antioxidant[9]. After calculating the IC_{50}, it was discovered that there are differences in the antioxidant activity between fermented kombucha for 12 days and the infusion of butterfly pea flowers. The results show that the fermented kombucha has a greater antioxidant activity at 19.54% compared to 23.33% for the butterfly pea flower infusion.

According to Essawet *et al*. fermented kombucha tea has a significant presence of antioxidants compared to non-fermented tea[10]. This increase in antioxidant activity is due to the presence of free phenolics during fermentation. The higher the phenolic content obtained, the higher the antioxidant levels[11]. Additionally, the change in antioxidant activity can be attributed to microbial activity during fermentation, which results in the production of organic acids that can act as antioxidants, such as ascorbic acid derived from d-saccharic acid 1,4-lactone (DSL) and Acetobacter xylinum through symbiosis between Gluconacetobacter sp. bacteria and lactic acid[12].

Table 1. The results of the antioxidant activity test of butterfly pea flower kombucha

Sample concentration (ppm)	Inhibition (%)	
	Butterfly pea flower infusion (unfermented)	Butterfly pea flower kombucha
50	9.55	24.11
100	20.58	33.01
150	31.61	41.91
200	42.64	50.80
250	53.67	59.70

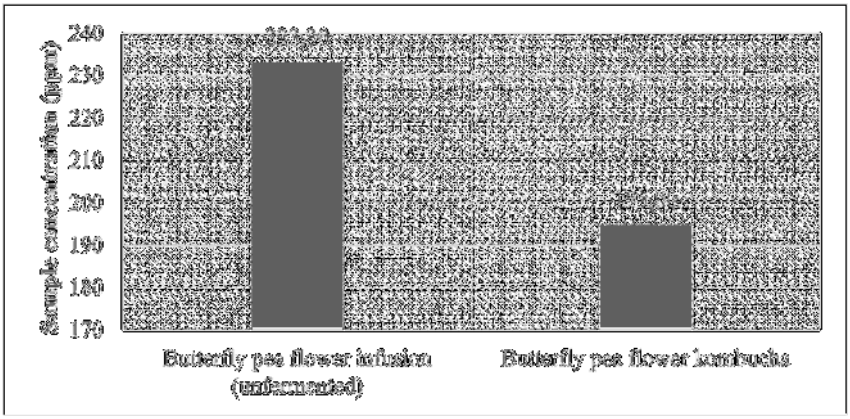

Figure 1. IC_{50} value of the antioxidant activity of butterfly pea flower infusion (unfermented) and butterfly pea flower kombucha

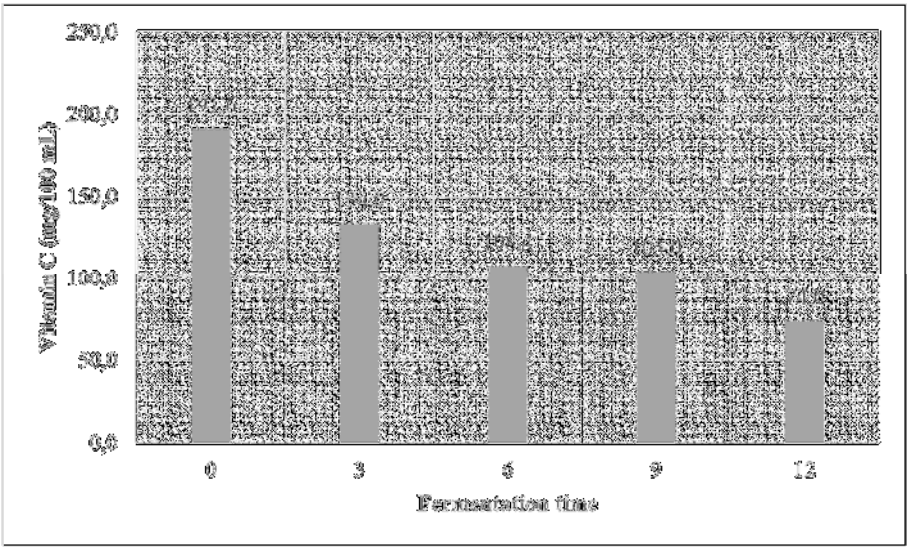

Figure 2. Vitamin C content during the butterfly pea flower kombucha fermentation.

The most common use of vitamin C is as an antioxidant. The graph in Figure 2. displays a declining trend in vitamin C concentration during fermentation. The degradation of vitamin C is affected by various factors such as temperature, oxygen, water activity, pH, and the presence of metal ions that work as catalysts. When it comes to fermentation processes like kombucha, the vitamin C content can decrease due to a combination of factors such as exposure to a temperature of 30°C for 15 days and the presence of oxygen[13]. This implies that the antioxidant properties in butterfly pea (*Clitoria ternatea* L.) kombucha are not derived from vitamin C but from the substances found in the phenolics of butterfly pea flowers and the microbial metabolism process during fermentation. Butterfly pea flowers contain various phenolic compounds, including anthocyanins, flavonoids, and tannins, which function as antioxidants. Based on research by Christiani and Lauda, the concentration of flavonoids in butterfly pea flowers increases during kombucha fermentation[14]. Primurdia and Kusnadi suggest that the increase in flavonoids concentration could be attributed to the activity of lactic acid bacteria, which break down complex phenolics by degrading sugars during fermentation and producing enzymes[15]. Phenolic compounds are released from the substrate, and flavonoid compounds are generated by adding phenolic groups to form flavonoids.

Conclusion

After being fermented for 12 days, kombucha has a very strong antioxidant activity, expressed as an IC_{50} value of 19.54%. However, the vitamin C content gradually decreases as the fermentation duration increases. In fact, the infusion's vitamin C content drops from 19.16 mg/100g to 7.40 mg/100g by the 12th day of fermentation.

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