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THE EFFECT OF TEMPERATURE AND FERMENTATION DURATION ON pH LEVEL AND VITAMIN C CONTENT OF BUTTERFLY PEA FLOWER (*Clitoria ternatea L.*) KOMBUCHA

Andy Anggoro Setyaji[1], Adita Silvia Fitriana[1*], Galih Samodra[1]

[1]Pharmacy Undergraduate Study Program, Universitas Harapan Bangsa

*Corresponding author: aditasilvia@uhb.ac.id

Abstract

Kombucha is produced through the fermentation of a tea and sugar mixture with a symbiotic culture of bacteria and yeast (SCOBY). It contains various compounds beneficial to health, including vitamin C. The pH level and vitamin C content of kombucha are affected by fermentation temperature and time. Correspondingly, this study aimed to determine the effects of temperature and fermentation duration on the pH level and vitamin C content of butterfly pea kombucha. The research was conducted employing laboratory experimental methods, including a UV-Vis spectrophotometer to measure vitamin C content and a digital pH meter to measure pH levels. The limit of detection (LoD) and the limit of quantification (LoQ) values for the UV-Vis spectrophotometer were 0.115 ppm and 3.77 ppm, respectively. The results indicated that fermentation temperature did not affect the pH or vitamin C content. However, the pH level was affected by fermentation time, while vitamin C content was not statistically affected. The highest pH value (5.11) was observed on day 0 of fermentation, and the highest vitamin C content (2.94%) was recorded on day 6 at a temperature of 30°C.

Keywords: *Clitoria ternatea, Fermentation, Kombucha, pH, Vitamin C*

Introduction

Kombucha is a functional beverage produced by microorganisms from bacterial and yeast families, collectively known as SCOBY, which ferment tea and sugar solutions. Kombucha tea can be made from various plants, including the butterfly pea flower, which can be used for brewing or fermenting into kombucha tea (Rezaldi et al., 2021). Kombucha contains various ingredients, such as organic acids, vitamin B, and vitamin C, which are associated with numerous health benefits (Purnami et al., 2018). Additionally, butterfly pea flowers are a source of vitamin C. Methanol extracts, water extracts, and infused water from the flowers contain vitamin C (Dianatasya, 2020).

Fermentation conditions, such as time and temperature, significantly affect the bioactive compounds generated, including vitamin C, which diminishes as the fermentation duration increases. This phenomenon occurs due to the insufficiency of the substrate needed for bacteria to synthesize vitamin C. Consequently, it cannot be formed and is converted into other acids (Wongthai et al., 2021).

Previous studies have demonstrated that the fermentation time could increase vitamin C levels in rosella kombucha up to day six, after which the levels could decrease on day nine and beyond

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(Winandari et al., 2022). Prior research has also demonstrated that higher fermentation temperatures result in increased levels of acids, metabolites, and vitamin C in kombucha tea (Aung & Eun, 2022; Winandari et al., 2022).

Kombucha has a sour taste due to the fermentation process. The pH value for safe consumption should not be lower than 3. When the pH value is lower, it is recommended to dilute kombucha with water, as an excessively tart flavor might potentially cause digestive issues (Nurhayati et al., 2020).

The pH value of kombucha is influenced by both the duration and conditions of fermentation. It decreases as fermentation time increases due to the rising acid concentration during the process. Previous studies on turmeric kombucha indicated that the pH value decreases over time because of the production of organic acids and secondary metabolites during fermentation (Wistiana & Zubaidah, 2015).

Fermentation temperature can also alter the pH value, as an increase in acid content causes a rise in the pH value. The optimum temperature for kombucha tea fermentation ranges from 20°C to 30°C. It is important to note that microorganisms play a crucial role in the fermentation process (Wongthai et al., 2021).

Based on the above discussion, understanding the fermentation conditions for butterfly pea flower kombucha is essential for achieving a kombucha with a safe pH value and high vitamin C content. Hence, the present study employed UV-Vis spectrophotometry for vitamin C quantification due to its advantages, including a low detection limit and high levels of accuracy and precision.

Methodology

Tools and Materials

The tools utilized in this research included electric stoves, stainless steel pans, spoons, filters, digital scales (And®), thermometers (Omron®), napkins, rubber bands, transparent glass jars, a UV-Vis spectrophotometer (Biobase®), glassware (Pyrex®), and digital pH meters (HANNA).

The materials used in this research included dried butterfly pea flowers, SCOBY, sugar, distilled water, and *L-ascorbic acid* (p.a).

Procedure

Kombucha starter production

Two liters of water were heated to 70°C. Subsequently, 10% sugar (w/v) was added and stirred until fully dissolved. The mixture was then cooled, and 0.5% (w/v) of butterfly pea flowers was added. The prepared kombucha starter was filtered and transferred into a 2 L glass jar containing a SCOBY culture. The jars were covered with fabric, secured, and left to ferment for 14 days at room temperature.

Butterfly pea kombucha production

Three liters of water were heated to 70°C. Subsequently, 0.5% (w/v) of butterfly pea flowers was added, and the stove was turned off. Then, 10% sugar (w/v) was added and stirred until fully dissolved. The mixture was cooled to 30°C, filtered to remove the pulp, and poured into three 1 L glass jars. 3% (w/v) of SCOBY and 10% (v/v) of kombucha starter were added to each jar. The jars containing butterfly pea flower tea and SCOBY were securely covered with fabric and fastened. The mixture was then fermented for 0, 3, 6, 9, 12, and 15 days at three different temperatures (18, 25, and 30°C).

Determination of kombucha's pH level

The pH meter electrode was immersed in the sample and left until a stable pH value was observed.

Determination of vitamin C content in kombucha

a. Preparation of ascorbic acid standard stock solution (100 ppm) (Saputri, 2018)

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0.5 mg of ascorbic acid was accurately weighed and transferred into a 100 mL measuring flask. This acid was then dissolved in distilled water and diluted to the specified volume mark.

b. Maximum wavelength determination

1 mL of the ascorbic acid standard stock solution was pipetted into a 10 mL measuring flask, and distilled water was added until the specified volume mark was reached. The absorbance of this solution was measured using a UV-Vis spectrophotometer across a wavelength range of 200–400 nm. A blank containing 2 mL of distilled water was placed in a separate cuvette. The wavelength corresponding to the highest absorbance was identified as the maximum wavelength of vitamin C.

c. Preparation of the ascorbic acid calibration curve (Chandra et al., 2019)

Volumes of 0.4 mL, 0.5 mL, 0.6 mL, 0.7 mL, 0.8 mL, and 0.9 mL of the ascorbic acid standard stock solution were pipetted into separate 10 mL measuring flasks. Distilled water was added to each flask until the volume reached the limit mark, creating a series of ascorbic acid standard solutions with concentrations of 4, 5, 6, 7, 8, and 9 ppm. The absorbance of these solutions was measured at the maximum wavelength.

d. Determination of vitamin C content in kombucha (Saputri, 2018)

A volume of 0.5 mL of kombucha solution was pipetted into a 10 mL measuring flask, and distilled water was added until the specified volume mark. This solution's absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength.

The sample absorbance was plotted on the calibration curve, and the vitamin C concentration in the sample was calculated using the linear regression equation:

$$y = bx + a \quad (1)$$

Subsequently, the vitamin C content in the sample was determined using the following formula:

$$\% \text{ content} = \frac{C \times V \times fp}{W} \times 100\% \quad (2)$$

C = concentration of vitamin C (mg/L)
V = volume (L)
fp = dilution factor
W = sample weight (mg)

e. Validation methods

1) Linearity

Linearity was assessed by creating a calibration curve of the ascorbic acid standard solution to evaluate the correlation between concentration and absorbance.

2) Accuracy

Accuracy was determined by calculating the percent recovery (% recovery) of ascorbic acid standard solutions using the formula:

$$\% \text{ recovery} = \frac{measured\ rate}{actual\ rate} \times 100 \quad (3)$$

3) Precision

Precision was evaluated by measuring the absorbance of an ascorbic acid standard solution six times at the maximum wavelength. The precision was expressed as the Relative Standard Deviation (% RSD):

$$SD = (2)\sqrt{\frac{\Sigma(X-\bar{X})^2}{n}} \quad (4)$$

$$RSD = \frac{SD}{\bar{x}} \times 100\% \quad (5)$$

SD = standard deviation
N = number of samples
x = sample concentration
$\bar{X}$ = average sample concentration

4) Limit of Detection (LoD) and Limit of Quantification (LoQ)

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LoD and LoQ were calculated using the following equations derived from the calibration curve:

$$LoD = \frac{SD \times 3}{b} \quad (6)$$

$$LoQ = \frac{SD \times 10}{b} \quad (7)$$

SD = standard deviation
b = slope of the linear regression equation

Results and Discussion

The plant used in this study was *Clitoria ternatea* L., commonly known as the butterfly pea. It is crucial to ensure the botanical identity of the plant materials to avoid errors during collection and to prevent the contamination of the main ingredients with other substances (Klau & Hesturini, 2021). The determination results, as confirmed by letter No. B/451/UN23.610/TA.00.01/2023, established that the plants under investigation were butterfly peas (*Clitoria ternatea* L.). The taxonomic classification of the butterfly pea is as follows: Family: *Fabaceae*; Genus: *Clitoria*; Species: *Clitoria ternatea*.

The measurement of pH values aimed to determine the acidity levels of butterfly pea flower kombucha at three different temperatures (18°C, 25°C, and 30°C) on specific days (0, 3, 6, 9, 12, and 15). Each measurement was repeated three times, as the data exhibited low variability, ensuring the repetitions were sufficient for accurate representation. The pH test on kombucha tea is essential for assessing its consumption safety. In this regard, a pH below 3 may cause digestive problems (Nurhayati et al., 2020). Moreover, the pH level influences the growth of microorganisms, such as yeast, in kombucha (Villarreal-Soto et al., 2018).

Kombucha is considered a non-toxic drink when prepared with proper knowledge and under hygienic conditions. The U.S. Food and Drug Administration and Kappa Laboratories in Miami, Florida, USA, have confirmed that kombucha is safe for human consumption (Jayabalan et al., 2016). However, no toxicity studies have been conducted specifically on butterfly pea kombucha, necessitating further research to evaluate its safety comprehensively.

Table 1. pH values of butterfly pea flower kombucha

Fermentation Time (days)	pH Value		
	18°C	25°C	30°C
0	5.11	5.11	5.11
3	3.30	3.16	3.10
6	3.26	3.11	3.02
9	3.18	3.02	2.94
12	3.11	2.94	2.86
15	3.02	2.86	2.78

The results in Table 1 portray that the pH value of butterfly pea flower kombucha gradually decreased from the start of fermentation (day 0) to day 15. The highest pH value (indicating lower acidity) was recorded on day 0 at 5.11, while the lowest (indicating higher acidity) was observed on day 15 at all fermentation temperatures. Prolonging the fermentation duration results in a reduction in pH levels. This is attributed to the yeast's ability to metabolize sugar into ethanol during fermentation, which is subsequently converted into organic acids by acetic acid bacteria, leading to a decrease in pH (Muttaqien, 2022).

Although ethanol levels were not measured in this study, previous research has demonstrated that ethanol levels in kombucha made from black tea and cascara coffee arabica increase to a maximum value before gradually decreasing as fermentation time increases (Chakravorty et al., 2016; Puspaningrum et al., 2022).

The maximum wavelength measurements of vitamin C were obtained by identifying the highest absorbance value within the 200–400 nm wavelength range. The maximum wavelength of vitamin C in this study was determined to be 266 nm, consistent with previous findings indicating a peak at the same wavelength (Rizkasari & Ismail, 2023). Measuring the maximum wavelength of vitamin C helps identify the

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substance most effectively absorbed by the UV-Vis spectrophotometer (Sukmawati et al., 2018).

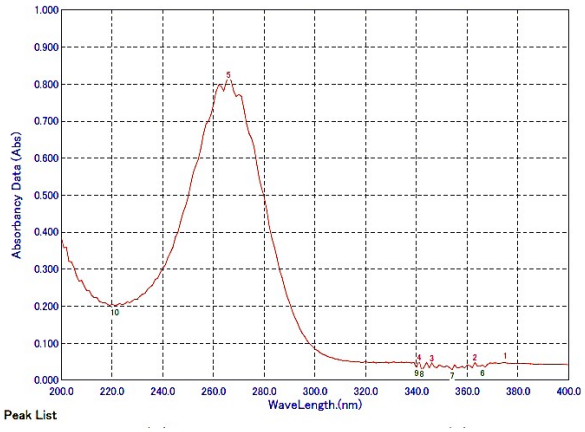

Figure 1. Maximum wavelength of Vitamin C

Vitamin C levels were calculated based on the standard curve, demonstrating a linear correlation between vitamin C concentration and absorbance. According to the standard curve, the linear regression equation y = 0.1006x + 0.1871 was obtained, with a correlation coefficient (r) of 0.9989.

The results indicated that a fermentation temperature of 30°C yielded the highest vitamin C level, measured at 2.94%. This could be attributed to the optimal growth range of *Saccharomyces cerevisiae* yeast at 30°C to 32°C (Parapouli et al., 2020). *Saccharomyces cerevisiae* facilitates the conversion of glucose into ethanol and carbon dioxide (CO_2). Ethanol, in turn, stimulates the growth of acetic acid bacteria, which produce acetic acid, maintaining acidic conditions that inhibit vitamin C degradation (Lonăr et al., 2006; Mushtaq et al., 2022). Consequently, on day 6, the vitamin C levels in kombucha fermented at 30°C exceeded those at 25°C and 18°C.

During fermentation, vitamin C levels decreased to 2.43% on the 15th day, following a peak on day 6. This decline was due to vitamin C degradation, influenced by factors such as temperature, light, enzymes, and oxygen (Wang et al., 2017). As a result, the vitamin C content diminished over time (Herlina & Muzdalifa, 2020).

The slight variation in vitamin C levels observed over the 15-day fermentation period might be attributed to oxygen entering the jar through the pores of the fabric covering it, leading to vitamin C oxidation. These findings aligned with previous research, which reported a decline in the vitamin C content of Rosella kombucha after the sixth day of fermentation at room temperature (Winandari et al., 2022).

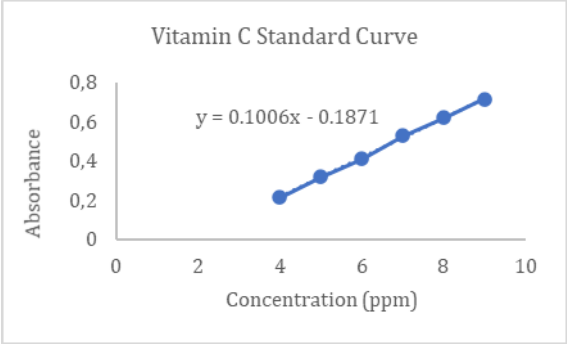

Figure 2. Standard curve of vitamin C

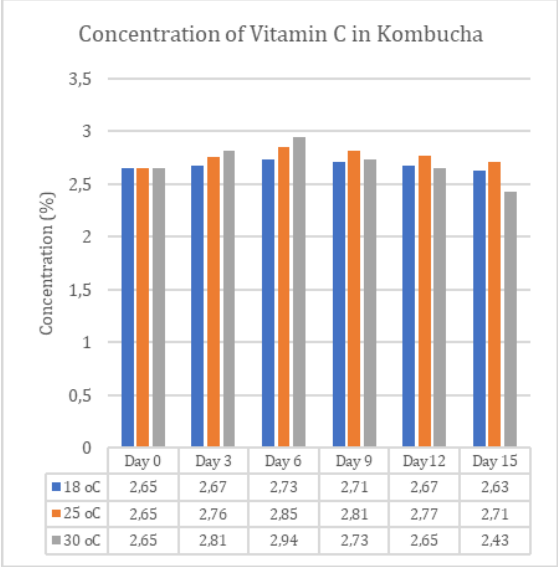

Figure 3. Vitamin C levels in Kombucha

The pH value and bioactive compounds produced during kombucha fermentation, including vitamin C, were influenced by various factors, such as the type of plant or tea used and fermentation conditions, including duration and temperature (Neffe-Skocińska et al., 2017). While *Camellia sinensis* is the most common raw material for kombucha, it is not conclusive that the vitamin C content of this

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kombucha is superior to that derived from other plants, as numerous factors influence the fermentation process.

Organoleptic observations revealed that butterfly pea kombucha underwent aroma, taste, and color changes during fermentation. By the 15th day, the kombucha had transformed from a sweet taste, characteristic butterfly pea flower aroma, and dark blue color to a very sour taste, a distinct sour kombucha aroma, and a bright purple color.

Accuracy tests were conducted to determine the proximity of analytical results to the actual analyte concentration, expressed as percent recovery. The test results revealed percent recovery values ranging from 98.9% to 101%, which fell within acceptable limits.

Table 2. Accuracy test results

Abs	Concentration (ppm)		% Recovery
	Actual	Measurable	
0.213	4	3.97	99.25%
0.318	5	5.02	100.40%
0.410	6	5.93	98.90%
0.528	7	7.10	101.00%
0.619	8	8.01	100.00%
0.713	9	8.94	99.30%

Precision was assessed by measuring the absorbance of a 6 ppm vitamin C solution in six replicates, categorized under repeatability. The precision test results yielded a standard deviation (SD) of 0.0466 and a coefficient of variation (CV) of 0.84%.

The Limit of Detection (LoD) and Limit of Quantification (LoQ) values were determined using the lowest vitamin C concentration (4 ppm). The LoD value was calculated as 1.13 ppm, while the LoQ was determined to be 3.77 ppm.

Table 3. Precision test results

Concentration (ppm)	Abs	X (ppm)
6	0.412	5.95
6	0.409	5.91
6	0.411	5.94
6	0.405	5.88
6	0.402	5.85
6	0.399	5.82
$\bar{X}$		5.89
SD		0.0466
CV		0.84%

Table 4. LoD and LoQ Test Results

Concentration (ppm)	Abs	X (ppm)
4	0.216	4.00
4	0.218	4.02
4	0.213	3.97
4	0.214	3.98
4	0.211	3.95
4	0.205	3.89
4	0.207	3.91
4	0.216	4.00
4	0.213	3.97
4	0.211	3.95
$\bar{X}$		3.96
SD		0.038
LoD		1.13
LoQ		3.77

Conclusion

Based on the results of the study, the following conclusions can be drawn:

1. The fermentation temperature (18°C, 25°C, and 30°C) did not affect the pH value and vitamin C content of butterfly pea flower kombucha. However, fermentation at 30°C yielded the lowest pH value (2.78) and the highest vitamin C content (2.94%).
2. The duration of fermentation (0, 3, 6, 9, 12, and 15 days) affected the pH value but did not affect vitamin C levels. The vitamin C content of kombucha tea underwent only minor changes during the fermentation process.

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References

Aung, T., & Eun, J. B. (2022). Impact of time and temperature on the physicochemical, microbiological, and nutraceutical properties of laver kombucha (Porphyra dentata) during fermentation. *LWT - Food Science and Technology*, *154*(2022), 1–9. https://doi.org/10.1016/j.lwt.2021.112643

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology*, *220*(2016), 63–72. https://doi.org/10.1016/j.ijfoodmicro.2015.12.015

Chandra, B., Zulharmita, Z., & Putri, W. D. (2019). Penetapan Kadar Vitamin C Dan B1 Pada Buah Naga Merah (Hylocereus Lemairel (Hook.) Britton & Rose) Dengan Metode Spektrofotometri Uv-Vis. *Jurnal Farmasi Higea*, *11*(1), 62–74. https://doi.org/10.52689/higea.v11i1.215

Dianatasya, A. (2020). *Analisa Kadar Vitamin C Infused Water Bunga Telang (Clitoria Ternatea) Dan Lemon (Citrus limon)(Studi di Perumahan Koala Regency Semolowaru Bahari, Surabaya*. STIKES Insan Cendekia Medika.

Herlina, & Muzdalifa, D. (2020). Pengaruh Suhu Penyimpanan Terhadap Kadar Vitamin C Buah Apel Merah (Pyrus malus L.). *Jurnal Penelitian Dan Kajian Ilmiah Kesehatan*, *6*(1), 119–127. https://jurnal.poltekmfh.ac.id/index.php/JPKIK/article/view/63

Jayabalan, R., Malbaša, R. V., & Sathishkumar, M. (2016). Kombucha. In *Reference Module in Food Science*. Elsevier.

Klau, M. H. C., & Hesturini, R. J. (2021). Pengaruh Pemberian Ekstrak Etanol Daun Dandang Gendis (Clinacanthus nutans (Burm F) Lindau) Terhadap Daya Analgetik Dan Gambaran Makroskopis Lambung Mencit. *Jurnal Farmasi & Sains Indonesia*, *4*(1), 6–12. https://doi.org/10.52216/jfsi. v4i1.59

Lonăr, E., Djurić, M., Malbaša, R., Kolarov, L. J., & Klašnja, M. (2006). Influence of working conditions upon Kombucha conducted fermentation of black tea. *Food and Bioproducts Processing*, *84*(C3), 186–192. https://doi.org/10.1205/fbp.04306

Mushtaq, M. W., Tariq, S., Hameed, A., Bashir, S., Bibi, S., Dogar, N. A., Batool, A., & Ishaq, S. (2022). Spectrophotometric Determination of Vitamin C in Underground Vegetables and Kinetic Modelling to Probe The Effect of Temperature and pH on Degradation of Vitamin C. *Pakistan Journal of Botany*, *54*(5), 1771–1775. https://doi.org/10.30848/PJB2022-5(12)

Muttaqien, G. P. A. (2022). *Karakteristik Sifat Fisikokimia Teh Kombucha Bunga Telang (Clitoria ternatea L.) Dengan Variasi Lama Fermentasi Dan Konsentrasi Starter SCOBY (Symbiotic Culture of Bacteria and Yeast)*. Universitas Muhammadiyah Malang.

Aung, T., & Eun, J. B. (2022). Impact of time and temperature on the physicochemical, microbiological, and nutraceutical properties of laver kombucha (Porphyra dentata) during fermentation. *LWT - Food Science and Technology*, *154*(2022), 1–9. https://doi.org/10.1016/j.lwt.2021.112643

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial

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and biochemical dynamics. *International Journal of Food Microbiology*, *220*(2016), 63–72. https://doi.org/10.1016/j.ijfoodmicro.2015.12.015

Chandra, B., Zulharmita, Z., & Putri, W. D. (2019). Penetapan Kadar Vitamin C Dan B1 Pada Buah Naga Merah (Hylocereus Lemairel (Hook.) Britton & Rose) Dengan Metode Spektrofotometri Uv-Vis. *Jurnal Farmasi Higea*, *11*(1), 62–74. https://doi.org/10.52689/higea.v11i1.215

Dianatasya, A. (2020). *Analisa Kadar Vitamin C Infused Water Bunga Telang (Clitoria Ternatea) Dan Lemon (Citrus limon)(Studi di Perumahan Koala Regency Semolowaru Bahari, Surabaya*. STIKES Insan Cendekia Medika.

Herlina, & Muzdalifa, D. (2020). Pengaruh Suhu Penyimpanan Terhadap Kadar Vitamin C Buah Apel Merah (Pyrus malus L.). *Jurnal Penelitian Dan Kajian Ilmiah Kesehatan*, *6*(1), 119–127. https://jurnal.poltekmfh.ac.id/index.php/JPKIK/article/view/63

Jayabalan, R., Malbaša, R. V., & Sathishkumar, M. (2016). Kombucha. In *Reference Module in Food Science*. Elsevier.

Klau, M. H. C., & Hesturini, R. J. (2021). Pengaruh Pemberian Ekstrak Etanol Daun Dandang Gendis (Clinacanthus nutans (Burm F) Lindau) Terhadap Daya Analgetik Dan Gambaran Makroskopis Lambung Mencit. *Jurnal Farmasi & Sains Indonesia*, *4*(1), 6–12. https://doi.org/10.52216/jfsi. v4i1.59

Lonăr, E., Djurić, M., Malbaša, R., Kolarov, L. J., & Klašnja, M. (2006). Influence of working conditions upon Kombucha conducted fermentation of black tea. *Food and Bioproducts Processing*, *84*(C3), 186–192. https://doi.org/10.1205/fbp.04306

Mushtaq, M. W., Tariq, S., Hameed, A., Bashir, S., Bibi, S., Dogar, N. A., Batool, A., & Ishaq, S. (2022). Spectrophotometric Determination of Vitamin C in Underground Vegetables and Kinetic Modelling to Probe The Effect of Temperature and pH on Degradation of Vitamin C. *Pakistan Journal of Botany*, *54*(5), 1771–1775. https://doi.org/10.30848/PJB2022-5(12)

Muttaqien, G. P. A. (2022). *Karakteristik Sifat Fisikokimia Teh Kombucha Bunga Telang (Clitoria ternatea L.) Dengan Variasi Lama Fermentasi Dan Konsentrasi Starter SCOBY (Symbiotic Culture of Bacteria and Yeast)*. Universitas Muhammadiyah Malang.

Neffe-Skocińska, K., Sionek, B., Ścibisz, I., & Kołożyn-Krajewska, D. (2017). Acid Contents and the Effect of Fermentation Condition of Kombucha Tea Beverages on Physicochemical, Microbiological and Sensory Properties. *CYTA - Journal of Food*, *15*(4), 601–607. https://doi.org/10.1080/19476337.2017.1321588

Nurhayati, N., Yuwanti, S., & Urbahillah, A. (2020). Karakteristik Fisikokimia Dan Sensori Kombucha Cascara (Kulit Kopi Ranum). *Jurnal Teknologi Dan Industri Pangan*, *31*(1), 38–49. https://doi.org/10.6066/jtip.2020.31.1.38

Parapouli, M., Vasileiadis, A., Afendra, A. S., & Hatziloukas, E. (2020). Saccharomyces cerevisiae and its industrial applications. In *AIMS Microbiology* (Vol. 6, Issue 1). https://doi.org/10.3934/microbiol.2020001

Purnami, K. I., Jambe, A. A., & Wisaniyasa, N. W. (2018). Pengaruh Jenis Teh Terhadap Karakteristik Teh Kombucha. *Jurnal Ilmu Dan Teknologi Pangan (ITEPA)*, *7*(2), 1–10. https://doi.org/10.24843/itepa.2018.v07.i02.p01

Puspaningrum, D. H. D., Sumadewi, N. L. U., & Sari, N. K. Y. (2022). Karakteristik Kimia dan Aktivitas Antioksidan

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Selama Fermentasi Kombucha Cascara Kopi Arabika (Coffea arabika L.) Desa Catur Kabupaten Bangli. *Jurnal Sains Dan Edukasi Sains*, *5*(2), 44–51. https://doi.org/10.24246/juses.v5i2p44-51

Rezaldi, F., Ningtyas, R. Y., Anggraeni, S. D., Ma'ruf, A., Fatonah, N. S., Pertiwi, F. D., Fitriyani, F., A, L. D., US, S., Fadillah, M. F., & Subekhi, A. I. (2021). Pengaruh Metode Bioteknologi Fermentasi Kombucha Bunga Telang (Clitoria ternatea L) sebagai Antibakteri Gram Positif dan Negatif. *Jurnal Biotek*, *9*(2), 169. https://doi.org/10.24252/jb.v9i2.25467

Rizkasari, G., & Ismail, R. (2023). Determination of Vitamin C Levels in Limes (Citrus aurantifolia Swingle) and Lemon (Citrus limon (L.) Burm. f.) Using the UV Vis Spectrophotometric Method. *Ad-Dawaa : Journal of Pharmacy*, *1*(2), 93–99. https://doi.org/10.52221/dwj.v1i2.415

Saputri, K. D. (2018). *Penetapan Kadar Vitamin C Pada Kubis (Brassicae oleracea var. capitata L.) Berdasarkan Ukuran Kecil Besar dan Sedang Secara Spektrofotometri UV*. Universitas Setia Budi.

Sukmawati, Sudewi, S., & Pontoh, J. (2018). Optimasi dan Validasi Metode Analisis Dalam Penentuan Kandungan Total Flavonoid Pada Ekstrak Daun Gedi Hijau (Abelmoscus Manihot L.) Yang Diukur Menggunakan Spektrofotometer Uv-Vis. *Pharmacon: Jurnal Ilmiah Farmasi - UNSRAT*, *7*(3), 32–41. https://doi.org/10.35799/pha.7.2018.20117

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J.-P., & Taillandier, P. (2018). Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*, *83*(3), 580–588.

Wang, J., Law, C. L., Mujumdar, A. S., Law, C.-L., & Xiao, H.-W. (2017). The Degradation Mechanism and Kinetics of Vitamin C in Fruits and Vegetables During Thermal Processing. In *Nema, PK, Kaur, BP and Mujumdar,(eds) AS Fundamentals & applications (Part III)* (pp. 227–253). https://www.researchgate.net/publication/319527724

Winandari, O. P., Widiani, N., Kamelia, M., & Rizki, E. P. (2022). Potential Of Vitamin C And Total Acid As Antioxidants Of Rosella Kombucha With Different Fermentation Times. *Jurnal Pembelajaran Dan Biologi Nukleus*, *8*(1), 141–148. https://doi.org/10.36987/jpbn.v8i1.2471

Wistiana, D., & Zubaidah, E. (2015). Karakteristik Kimiawi dan Mikrobiologis Kombucha dari Berbagai Daun Tinggi Fenol Selama Fermentasi. *Jurnal Pangan Dan Agro Industri*, *3*(4), 1446–1457.

Wongthai, N., Tanticharakunsiri, W., Mangmool, S., & Ochaikul, D. (2021). Characteristics and antioxidant activity of royal lotus pollen, butterfly pea flower, and oolong tea kombucha beverages. *Asia-Pacific Journal of Science and Technology*, *26*(4), 1–11. https://doi.org/10.14456/apst.2021.47

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