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The Effect of Butterfly Pea Flower (*Clitoria ternatea*) Extract Addition on Total Dissolved Solids, Yeast Count, Total Acidity, and Organoleptic Properties of Water Kefir

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Abstract

This study aims to determine the effect of varying concentrations of butterfly pea flower extract (*Clitoria ternatea*) on kefir water in terms of total dissolved solids (TDS), total acidity, yeast count, and organoleptic properties. The materials used were dried butterfly pea flowers dissolved in mineral water (extract) and ingredients for producing kefir water. The experiment was conducted using a Completely Randomized Design (CRD) with extract concentrations of 0%, 0.5%, 1.0%, and 1.5% (v/v), each repeated five times. Data were analyzed using Analysis of Variance (ANOVA) and followed by Duncan's Multiple Range Test (DMRT) for the parameters of total dissolved solids, acidity, and yeast count. Organoleptic tests were analyzed using the non-parametric Kruskal-Wallis test, and if significant differences were found, they were followed by the Mann-Whitney test. The results of the study showed that the addition of butterfly pea flower extract at different concentration variations significantly affected ($p<0.05$) total dissolved solids, total acidity, and total yeast, with average values of total dissolved solids ranging from 10.90 to 10.40°Brix, total acidity from 0.05 to 0.21%, and total yeast of 1.60×10^6 CFU/ml to 1.30×10^6 CFU/ml. However, it did not affect taste, aroma, and texture, although color showed significant differences ($p<0.05$). Organoleptic test results yielded taste scores of 2.72 - 2.84, color scores of 1.44 - 4.88, aroma scores of 2.36 - 2.92, and texture scores of 2.04 - 2.36. However, the addition of butterfly pea flower extract at varying concentrations tended to reduce total soluble solids while increasing yeast count, total acidity, and enhancing color attributes in sensory evaluation.

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Introduction

Public awareness of the importance of maintaining good health continues to grow, influencing changes in consumption patterns-especially in the selection of foods and beverages that offer added health benefits. Today's consumers prioritize not only flavor but also the health advantages a product can provide (Anggoro et al., 2022). One of the current trends gaining popularity is the consumption of probiotic fermented beverages, which are recognized for supporting digestive health and boosting the immune system (Astuti et al., 2018). Water kefir is one such fermented drink that is increasingly favored due to its beneficial probiotic content.

Water kefir is produced by fermenting sugar water using cultures of probiotic-rich microorganisms, making it a favorable beverage for digestive health. It is also an excellent alternative for individuals who wish to enjoy the benefits of probiotics without consuming dairy-based products (Anggoro et al., 2022). Butterfly pea flower contains various chemical compounds such as tannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, stigmast-4-ene-3,6-dione, essential oils, and steroids (Al-Sanafi, 2016). These compounds are known to have multiple benefits, including antioxidant, antimicrobial, antiparasitic, anticancer, anti-allergic, and other health-promoting properties. The antioxidant activity of butterfly pea flower extract is considered strong, with an IC50 value of 87.86 ppm (Cahyaningsih et al., 2019). This makes butterfly pea flower a promising ingredient for functional and nutraceutical food products (Marpaung, 2020).

In developing water kefir with butterfly pea flower extract, several key parameters must be considered to ensure product quality and consumer acceptance. These include total dissolved solids (TDS), which indicate sugar content; total acidity, which influences flavor; and yeast count, which affects aroma. The addition of butterfly pea extract, rich in bioactive compounds, can alter these parameters. Moreover, organoleptic properties—especially the natural purple color from anthocyanins—play a vital role in enhancing the product's visual appeal and consumer preference.

It is essential to ensure that the concentration of butterfly pea flower extract does not disrupt the distinctive flavor and aroma balance of water kefir. While several studies have explored natural additives in water kefir, research specifically on butterfly pea extract remains limited. Studies by Imassilawati (2022) and Prasetyo (2024) examined its chemical and sensory impacts but lacked clarity on optimal concentrations. Furthermore, prior research has focused more on chemical properties than sensory aspects like taste, color, and aroma. Therefore, further research is needed to assess the effects of varying concentrations of butterfly pea extract on total soluble solids, acidity, yeast count, and organoleptic properties. The goal is to develop an optimal formulation that is both functionally beneficial and sensory appealing, supporting the development of functional foods rooted in Indonesian local wisdom.

Materials and Methods

This research was conducted from November to December 2024 at the Food Chemistry and Nutrition Laboratory and the Food Engineering Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang.

Materials

The materials used in this study included commercially available dried butterfly pea flowers purchased online, mineral water, sucrose (granulated sugar), and water kefir grains as the base ingredients for preparing butterfly pea water kefir. Other materials used for analysis included distilled water, butterfly pea water kefir samples, 0.85% physiological NaCl solution, Potato Dextrose Agar (PDA) media, 1% phenolphthalein (PP) indicator, 0.1 N NaOH, ethanol, and organoleptic evaluation questionnaires.

The equipment used in this study included: The equipment used in this study included: a grinder, 60-mesh sieve, hand refractometer, yeast incubator (30℃), sterilization oven, autoclave, burettes, laminar airflow cabinet, and organoleptic test questionnaires.

Methods

The preparation of butterfly pea flower extract was conducted based on the method by Fangohoi et al. (2023), with modifications and preliminary research as reference. The extraction process began by grinding dried butterfly pea flowers using a grinder until they turned into fine powder, which was then sieved using a 60-mesh filter. The resulting powder was dissolved in mineral water with a ratio of 5:1 (mineral water to powder), as determined through preliminary experiments. Mineral water was gradually added until the mixture could be stirred and filtered (no longer paste-like), achieving the required consistency. The mixture was then filtered using a filter cloth. The filtered extract was pasteurized using a hot plate for 30 minutes at a temperature of 60°C. After pasteurization, the extract was allowed to cool at room temperature (25°C) before use.

Preparation of Butterfly Pea Water Kefir

The next step was the preparation of water kefir with varying concentrations of butterfly pea extract, based on the modified method of Hastuti and Kusnadi (2016) and preliminary research. The process started with the preparation of a sugar solution by dissolving 5 litres of sucrose solution at a concentration of 12.5% (b/v) on a hot plate at 80°C until fully dissolved. Once the solution reached room temperature (±25°C), 250 mL was poured into each plastic jar for the treatment groups. The butterfly pea extract, also at room temperature, was then added to each jar at concentrations of 0% (P0), 0.5% (P1), 1.0% (P2), and 1.5% (P3) (v/v) of the 250 mL sugar solution. Next, water kefir grains were added at 5% of the total volume (250 mL) and gently stirred until evenly mixed. The fermentation process was carried out anaerobically at room temperature (25°C) for 24 hours. After fermentation, the kefir was filtered using a strainer to separate the grains from the fermented liquid. This process was repeated for five replications.

The selected extract concentration treatments were determined based on preliminary research, where visual inspection of color differences was used to evaluate additions of 1% to 8% butterfly pea extract. The resulting samples were tested for total dissolved solids, yeast count, total acidity, and organoleptic properties.

Data Processing and Analysis

The data obtained from the measurements of total dissolved solids, yeast count, and total acidity were analyzed statistically using Analysis of Variance (ANOVA) at a 5% significance level ($\alpha = 0.05$). If a significant effect ($p<0.05$) was observed, Duncan's Multiple Range Test (DMRT) was used as a post hoc test to identify differences among treatments. Organoleptic test data were analyzed using the non-parametric Kruskal-Wallis test and, if significant differences were found, followed by the Mann-Whitney test. All data analyses were performed using the Statistical Product and Service Solutions (SPSS) version 27.0 for macOS. The yeast count was determined using the Total Plate Count (TPC) method in the range of 30–300 CFU/mL, and the data was log-transformed before analysis and incubated inversely at 30°C for 48 hours.

Results and Discussion

Total Dissolved Solids (TDS)

The results of the water kefir tests with various concentrations of butterfly pea flower extract for the TDS parameter are shown in Table 1. Based on the data, the highest TDS was found in the 0% extract treatment (P0), while the lowest was observed at 1.5% (P3). This indicates that different concentrations of butterfly pea flower extract had a significant effect ($p<0.05$) on the TDS of the resulting water kefir. The

Table 1. Test Results of Total Dissolved Solids, Yeast Count, and Total Acidity of Butterfly Pea Water Kefir

Treatment	Butterfly Pea Extract Concentration			
	P0 (0%)	P1 (0.5%)	P2 (1.0%)	P3 (1.5%)
TDS (°Brix)	10.90 ± 0,000^{a}	10.70 ± 0,000^{b}	10.50 ± 0,000^{c}	10.40 ± 0.000^{d}
Yeast count (CFU/ml)	$1.6 \times 10^{6\ a}$	$2.4 \times 10^{6\ b}$	$1.9 \times 10^{6\ ab}$	$1.3 \times 10^{6\ a}$
Total acidity (%)	0.05 ± 0,000^{a}	0.09 ± 0,000^{b}	0.14 ± 0.018^{c}	0.21 ± 0.027^{d}

Results are mean±standard deviation (n = 15); No superscript letters shown, as no significant differences were found (p>0.05).

standard deviation for all treatments was zero, suggesting that all values were identical to their mean (Febriani, 2022). This was consistent across all experiments, from the trial and error phase to data collection.

TDS is a parameter used to assess sugar content—here, sucrose—and influences sweetness, flavor, and overall sensory properties of the water kefir with added extract (Sintasari et al., 2014; Simanjuntak et al., 2017). There is a noticeable tendency for TDS to decrease as the concentration of butterfly pea extract increases. This is attributed to the acidic nature of the butterfly pea flower, which facilitates the creation of an acidic environment that promotes the activity of lactic acid bacteria and yeasts during fermentation. Butterfly pea has an optimal pH range of approximately 5 to 6 (Sumartini, 2020). The higher acidity level of the extract enhances the stability of its color compounds, as the pigments in butterfly pea tend to be more stable in acidic solutions compared to neutral or alkaline ones (Husna et al., 2022).

Moreover, the addition of water during the preparation of butterfly pea extract results in an increased total volume, which consequently dilutes the concentration of dissolved solids. Thenu et al. (2023) reported that an increase in water content in food materials leads to a decrease in total dissolved solids. A higher dilution ratio corresponds to a lower TDS value due to the increased amount of solvent. Additionally, the incorporation of water affects the taste, consistency, and concentration of the butterfly pea extract. Rakhmawati and Yunianta (2015) found that increasing the fruit-to-water ratio up to 1:6 significantly reduced the TDS content in ambarella fruit juice. According to the Indonesian National Standard (1992), no specific TDS standard exists for water kefir, but reference can be made to yogurt, which requires a minimum of 8.2% TDS. Therefore, the values in this study meet this standard.

Although water kefir and yoghurt are produced using different substrates, they share a core similarity as lactic acid bacteria (LAB)-driven fermented products. Yoghurt has long-established international standards, particularly outlined in the Codex Alimentarius (FAO/WHO, 2011), which specify parameters such as final pH, LAB count, and total solids content. These parameters serve as a benchmark for evaluating fermentation efficiency, microbial viability, and product stability, which are also critical aspects in the development of water kefir.

Yeast Count

Based on the data presented in Table 1, the highest yeast count was observed in treatment P1, which involved the addition of 0.5% butterfly pea extract, while the lowest count occurred in treatment P3 with a 1.5% extract concentration. This indicates that varying concentrations of butterfly pea extract significantly affected the total yeast population ($p < 0.05$). A trend was observed in which yeast counts initially increased and subsequently decreased as the concentration of the extract increased.

The initial increase may be attributed to the acidic nature of butterfly pea, which facilitates the creation of an acidified environment favorable for microbial growth. Anthocyanins in butterfly pea are known to exhibit high stability and deep blue-purple coloration at pH levels between 4 and 5 (Angriani, 2019). Conversely, the decline in yeast population at higher concentrations could be associated with the presence of tannins, which possess antimicrobial properties. Butterfly pea contains various secondary metabolites including saponins, flavonoids, tannins, and terpenoids (Purwaniati et al., 2020). Flavonoids and tannins are known for their antioxidant, antimicrobial, and antifungal properties (Sumi et al., 2020), while saponins demonstrate anti-inflammatory, antifungal, antibacterial, antiparasitic, anticancer, and antiviral activities (Chatri et al., 2022). Terpenoids have been identified as potential anticancer agents (Cox-Georgian et al., 2019).

The bioactive compounds present in butterfly pea suggest its potential as a natural antifungal alternative. Jayadi et al. (2022) reported that flavonoids could inhibit fungal proliferation by binding to microtubule proteins, disrupting spindle formation, and slowing mitosis (Nguyen et al., 2021). However, in this study, no inhibition zones were observed in the treatment groups, indicating limited antifungal efficacy at the tested concentrations.

Statistically significant differences were observed between treatment P0 and P1, while treatments P2 and P3 did not differ significantly, as indicated by distinct superscripts. The total yeast counts ranged from 1.3×10^6 to 2.9×10^6 CFU/mL. Since Codex Alimentarius (2003) does not specify yeast count standards for water kefir, the minimum threshold established for fermented milk products such as yogurt ($\geq 1.0 \times 10^4$ CFU/mL) was used as a reference. Accordingly, all treatments in this study met the Codex yeast count standards.

Total Acidity

The addition of butterfly pea flower extract significantly affected total acidity ($p<0.05$), with the highest value at 1.5% (P3) and the lowest at 0% (P0). There was a clear trend of increasing acidity with higher extract concentrations. Since no official acidity standard exists for water kefir, yogurt guidelines are referenced, which suggest lactic acid levels between 0.5–2.0% (SNI, 1992), while Codex (2003) sets a minimum of 0.6% for milk kefir. The total acidity in this study ranged from 0.05% to 0.21%, indicating it does not meet the SNI or

Codex standards.

The increase in acidity is due to the acidic nature of the butterfly pea flower (pH 4–5), which supports microorganism growth (Angriani, 2019). The low acidity values may result from a mismatch between substrate type and yeast growth requirements, which influence enzyme activity and productivity (Amalia et al., 2022). Six main factors affect yeast activity in food: moisture, oxygen concentration, temperature, nutrient availability, pH, and the presence of inhibitors. Nutrient availability depends on the composition of the medium, and each yeast species has different capabilities to metabolize proteins, carbohydrates, and fats (Yurliasni & Zakaria, 2014).

Organoleptic Properties

Organoleptic testing was conducted by placing 30 ml of butterfly pea flower water kefir sample in plastic cups that had been randomly assigned three-digit codes to reduce assessment bias. A total of 25 semi-trained panelists were given questionnaires that included instructions, panelist responses, and testing information, such as panelist names, dates, signatures, and samples tested. Drinking water was provided to neutralize the mouth between sample changes.

The attributes evaluated included texture, taste, color, and aroma, with ratings using a 1–5 scale, where taste was rated as 1 = very strong water kefir flavor, 2 = strong water kefir flavor, 3 = somewhat strong water kefir flavor, 4 = slightly strong water kefir flavor, and 5 = very little water kefir flavor. For aroma, 1 = very aromatic water kefir, 2 = aromatic water kefir, 3 = slightly aromatic water kefir, 4 = not aromatic water kefir, and 5 = very not aromatic water kefir. The color scale is 1= very not purple, 2= not purple, 3= slightly purple, 4= purple, 5= very purple, and the texture scale is 1= very thin, 2= thin, 3= slightly thin, 4= not thin, and 5= very not thin. Kruskal-Wallis and Mann-Whitney tests showed no significant differences ($p>0.05$) in taste, aroma, or texture between P0 - P3, but there was a significant difference in color ($p<0.05$).

Table 2. Sensory Evaluation Results for the Taste Attribute of Butterfly Pea Water Kefir

Treatment	Taste Score	Rounded	Description
P0 (0%)	2.72±1.40	3	Slightly tasted water kefir
P1 (0.5%)	2.56±0.65	3	Slightly tasted water kefir
P2 (1%)	2.84±1.11	3	Slightly tasted water kefir
P3 (1.5%)	2.84±1.60	3	Slightly tasted water kefir

Results are mean±standard deviation (n = 15); No superscript letters shown, as no significant differences were found ($p>0.05$).

Taste

As shown in Table 2, different concentrations of extract did not significantly affect taste ($p>0.05$). The addition of butterfly pea flower extract did not significantly affect taste perception, as all treatments received a score of 3, indicating a slight water kefir taste. This is also due to the characteristics of butterfly pea flower extract, which does not give a strong or distinctive taste to water kefir. In general, the use of butterfly pea flower extract does not affect the taste, aroma, or flavor of dishes, because butterfly pea flowers only contain anthocyanin pigments (Purba, 2020).

Table 3. Sensory Evaluation Results for the Color Attribute of Butterfly Pea Water Kefir

Treatment	Color Score	Rounded	Description
P0 (0%)	1.44±0.51^{a}	1	Not purple at all
P1 (0.5%)	2.92±0.28^{b}	3	Slightly purple
P2 (1%)	3.96±0.20^{c}	4	Purple
P3 (1.5%)	4.88±0.33^{d}	5	Very purple

Results are mean±standard deviation (n = 15); No superscript letters shown, as no significant differences were found ($p>0.05$).

Color

Color attributes differed significantly ($p<0.05$) among treatments. The mean score increased with extract concentration, from "not purple" to "very purple," confirming that extract concentration directly enhanced color intensity. The purple color comes from anthocyanins, which appear purple in acidic solutions. These pigments are flavonoids with antioxidant activity that protect against free radicals (Endang, 2020). More extract resulted in a deeper purple color.

Table 4. Sensory Evaluation Results for the Aroma Attribute of Butterfly Pea Water Kefir

Treatment	Aroma Score	Rounded	Description
P0 (0%)	2.36±1.25	2	Water kefir aroma
P1 (0.5%)	2.56±0.65	3	Slight water kefir aroma
P2 (1%)	2.60±1.00	3	Slight water kefir aroma
P3 (1.5%)	2.92±1.55	3	Slight water kefir aroma

Results are mean±standard deviation (n = 15); No superscript letters shown, as no significant differences were found ($p>0.05$)..

Aroma

In terms of aroma attributes, no significant differences ($p>0.05$) were found in the aroma of water kefir between treatments. This indicates that butterfly pea flower extract does not affect the aroma of water kefir, suggesting that the dominant aroma of water kefir tends to mask the influence of butterfly pea flower extract. The aroma produced in this beverage formulation is the characteristic fresh acidic aroma from the fermentation process (Khalisa et al., 2021). Hibiscus flowers are one type of flower that does not have a strong aroma (El-Hamshary et al., 2022). The use of butterfly pea flower extract does not affect the aroma or taste of food or beverages, as this extract only contains anthocyanins, which are natural pigments (Ramadani et al., 2023).

Texture

Table 5 shows no significant differences ($p>0.05$) in texture among treatments. Texture is a tactile sensation during chewing or swallowing and is a crucial factor in consumer acceptance (Nurlita, 2017; Rahmawati et al., 2024). The addition of extract did not affect texture, and the product remained consistently watery. Unlike milk kefir, water kefir lacks proteins that coagulate in acidic conditions, resulting in a thinner consistency (Mulyani et al., 2021).

Table 5. Sensory Evaluation Results for the Texture Attribute of Butterfly Pea Water Kefir

Treatment	Texture Score	Rounded	Description
P0 (0%)	2.04 ± 1.02	2	Watery
P1 (0.5%)	1.84 ± 0.63	2	Watery
P2 (1%)	2.16 ± 0.63	2	Watery
P3 (1.5%)	2.36 ± 0.76	2	Watery

Results are mean±standard deviation (n = 15); No superscript letters shown, as no significant differences were found (p>0.05).

Conclusion

The results of the study indicate that the atabddition of butterfly pea flower extract at different concentrations in the production of water kefir affects total dissolved solids, total yeast, total acidity, and color aspects in organoleptic testing. The addition of butterfly pea flower extract tends to reduce total dissolved solids but increases total yeast, total acidity, and color attributes in organoleptic testing, as evidenced by the fact that the higher the concentration of butterfly pea flower extract, the more intense the purple color formed. The best treatment was obtained by adding 1.5% butterfly pea flower extract in the production of water kefir.

Recommendations

Based on the results of this study, it is recommended to thoroughly understand the substrate used to ensure optimal microbial growth. In addition, further research needs to investigate in depth the characteristics and properties of butterfly pea flowers related to food ingredients to avoid formulation errors and potential negative impacts on the resulting butterfly pea water kefir, such as the loss of antioxidant properties when exposed to heat.

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