



Formulation and evaluation of kombucha with butterfly Pea

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Abstract

Kombucha is a fermented tea beverage renowned for its probiotic content, antioxidant properties, and potential health benefits, particularly in supporting gut health and improving digestion. In this study, butterfly pea flower (*Clitoria ternatea*) was incorporated into kombucha to enhance its functional and aesthetic qualities. Butterfly pea is known for its rich content of anthocyanins, flavonoids, and other bioactive compounds that exhibit antioxidant, anti-inflammatory, and neuroprotective effects. The infusion of butterfly pea into kombucha not only contributes additional nutritional value but also results in a striking visual transformation, producing a vibrant blue to purple color depending on the pH level during fermentation.

The combination of kombucha and butterfly pea creates a synergistic beverage that appeals to health-conscious consumers seeking natural, functional drinks. The fermentation process was observed to maintain the bioactive properties of both components while enhancing the flavor profile with mild floral and earthy notes. This study highlights the potential of butterfly pea kombucha as a novel, health-promoting beverage with commercial viability in the functional food and beverage market. Further research on its shelf life, microbial composition, and specific health effects could support its development as a mainstream wellness product, contributing to the diversification of probiotic-rich drinks.

Keywords:

Kombucha, Butterfly Pea, Fermented Beverage, Probiotics, Antioxidants, Functional Drink, Natural Colorant, Gut Health.

Introduction

Kombucha is a naturally fermented tea beverage that has gained global attention for its unique flavor profile and potential health benefits, including probiotic activity, antioxidant properties, and support for gut health. Traditionally made by fermenting sweetened black or green tea with a symbiotic culture of bacteria and yeast (SCOBY), kombucha has become a platform for innovation in the functional beverage industry. [1,2]

A great deal of positive testimonial and anecdotal tribute has been expressed regarding the regular consumption of Kombucha (Japanese, “kombu;” “cha, “), and its effects on physiological well-being.

Kombucha, a popular fermented tea, and secondary fermentation of butterfly Pea owes its distinctive taste and purported health benefits to a symbiotic culture of bacteria and yeast (SCOBY). This complex fermentation process produces a beverage rich in probiotics, organic acids, and antioxidants. With the rise of kombucha’s popularity, ensuring its safety, consistency, and compliance with regulatory standards has become increasingly important.[1,3,5] Inconsistent microbial profiles or contamination can lead to quality issues or even health risks.

Nowadays people are increasingly concerned about their own health. People want to become healthier, and the adoption of healthier lifestyles is necessary to improve quality of life and to reduce the numerous pathologies and comorbidities associated with nutrient inadequacy or poor diet choices [2,3] People are concerned about their body, their mind and their health, and so their search for healthy foods or specific foods that improve health, such as functional foods, has been increasing

Kombucha, a fermented tea drink, meets the vibrant blue hue and potential health benefits of butterfly pea (*Clitoria ternatea*). This innovative blend combines the probiotic-rich goodness of kombucha with the antioxidant and anti-inflammatory properties of butterfly pea, creating a unique and refreshing beverage experience.[4,6]

Benefits:

Kombucha with butterfly pea flower is a beautiful and powerful combination both for health and appearance.

1. Rich in Antioxidants: Kombucha is already packed with antioxidants from the tea and the fermentation process. Butterfly pea flowers are especially rich in anthocyanins (the pigments that make them blue/purple), which help fight oxidative stress and protect your cells.[1,20,33]

2. Gut Health Support: The probiotics from kombucha help balance your gut microbiome, improving digestion, immunity, and even mental health (gut-brain connection).

3. Anti-Inflammatory Properties: Butterfly pea contains natural anti-inflammatory compounds, which can help reduce chronic inflammation and support general wellness.

4. Blood Sugar Regulation: Some studies suggest butterfly pea extract may help regulate blood sugar levels, and kombucha (especially when low in sugar) also helps in moderating insulin response.

5. Mood and Stress Relief: Butterfly pea has been traditionally used for calming the mind, reducing anxiety, and even mildly boosting mood.

Plus, kombucha can also help indirectly through gut health, since much of your serotonin (happy chemical) is made in the gut.

6. Cognitive Support: Butterfly pea is believed to enhance memory and brain function — partly due to its antioxidant load and traditional use in Ayurveda and Thai medicine

7. Ease pain and migraine 8. accelerates ulcer healing 9. enhance digestion and bowel function[44]

■ Five ways that drinking reduces stress:

- Adrenaline and cortisol are released to speed up heart rate, arouse senses, and prime muscles for rapid action.

1. Beverages are adaptogen: An adaptogen is defined as a drug, usually a herb that by restoring physiological function and reducing stress response, improves the body's

capability to withstand stressors, boost vitality, and improve work capacity and concentration.

2. Drink serves as a stabilizing a healthy digestive system.: Improved digestion and acidity can help with ulcers and irritable bowel syndrome, two common gut manifestations of stress.[44,32]

3. Has vitamin C and B vitamins.: The presence of vitamin B6 and B12, often known as thiamine, in this product is proven to aid the body combat depression, balance mood, and enhance concentration. Includes vitamin C as well, which inhibits the release of cortisol, one of the stress hormones. Elevations of cortisol in the blood can lead to depression, hypertension, and difficulty thinking clearly.

4. Drinking can lower sugar and caffeine intake.: Tea's L-theanine balances the negative effects of caffeine, giving you calm, concentrated energy.

5. Moderate alcohol use is good for the body Although it is not an alcoholic ferment.: Such as wine or beer, this beverage does contain traces of alcohol. These naturally occurring low alcohol concentrations can raise and stress lower emotions of wellbeing.[1,2,23].

Material & method

Collection of Ingredient

Kombucha, a fermented tea drink, meets the vibrant blue hue and potential health benefits of butterfly pea (*Clitoria ternatea*). This innovative blend combines the probiotic-rich goodness of kombucha with the improve gut health, antioxidant and anti-inflammatory properties of butterfly pea, creating a unique and refreshing beverage experience.

Butterfly pea extraction :

To make an extract of butterfly pea flower for secondary fermentation, 3 gram of Dried flowers in a 30ml of boiling water (5 minutes), until the water turns a vibrant blue color, remove the flowers and cool.

Table 1. information about Ingredient

		
<p>Fig.1: SCOBY</p> <p>SCOBY, or Symbiotic Culture of Bacteria and Yeast, is a gelatinous, rubbery substance used in the fermentation of beverages like kombucha. It's a living culture</p> <p>Made of bacteria and yeast that work together to convert sweetened tea into kombucha. SCOBYs are essential for producing the characteristic sour, fizzy flavour of kombucha[1,3,7]</p>	<p>Fig.2: tea</p> <p>Tea varieties include: black, white, green, oolong, and pu-erh . Black tea is a popular choice for making kombucha because it provides a robust flavour and a good source of nutrients for the SCOBY (Symbiotic Culture of Bacteria and Yeast).</p> <ol style="list-style-type: none"> 1.Strong flavour profile: Black tea's robust taste. 2.Nutrient-rich: Black tea contains compounds that support the growth of the 	<p>Fig.3: sugar</p> <p>Sugar varieties include: Evaporated cane juice , white sugar, pasteurized honey, unrefined cane sugar/brown sugar .</p> <p>For effective fermentation, kombucha needs sucrose-rich sugars. Sucrose is transformed into glucose and fructose, which are directly used by the microorganisms to produce acids and gas, essential for fermentation.[5,6]</p>

	SCOBY and the fermentation process.[2,3]	
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Fig.4: starter

Kombucha Starter liquid is healthy, aged kombucha that contains the living bacteria and yeast needed to brew kombucha. This liquid kombucha culture is usually taken from a previous batch of kombucha and is then used to kick start a new batch of kombucha, hence the name starter tea.[1,4]



Fig.5: Butterfly pea

Butterfly pea also known as *Clitoria ternatea*, is a flowering plant in the Pea family (Fabaceae) native to South Asia. It's recognized for its vibrant blue flowers.

- Scientific name: *Clitoria ternatea*. ,,
- Common names: Butterfly pea, blue pea, Asian pigeonwings, bluebellvine, etc.
- Appearance: It's a vine with distinctive blue flowers that can be brewed into a tea[30]

■ Equipment used for the fermentation process

Includes a fermenter vessel, a strainer, a long-handled spoon, a cloth cover, a thermometer, a filter, a funnel, a digital balance, a PH strip or PH meter, and a measuring cylinder.

■ Formulation table

Table 2: Primary fermentation**

Vessel size	Tea bags	Sugar	Scoby	Starter liquid	Brew cycle (days)
½ gallon	2-3 (1 tablespoon)	6 tablespoons	1 small	½-1 cup	3-7
1 gallon	3-5 (1-2 tablespoons)	½ cup	1 large *	1 cup	7-14
1½ gallons	4-6 (1-2 tablespoons)	1 cup	1 large	1 cup	7- 21

Table3: Secondary fermentation*

Dried Butterfly Pea (gm)	Glass bottle (ml)
2	200
3	300
4	400
5	500 (0.5 litre)

■ Fermentation process

Primary fermentation :

Step 1: Preparation of tea

Dip the 3-5 black tea bags in 3 litre of boiled water of 5 minutes, remove the tea bags

step 2: add the ½ cup sugar to the hot tea stir the liquid until completely dissolved then cool to the fermentation process.

Step 3: Pour the tea in brewing vessel. To check a temperature (about 100° F [38° C]) , cover with a clean cloth and set aside until lukewarm

Step 4: with clean hands, place the SCOBY in sweet tea solution. Pour the starter liquid on top of the SCOBY

Step 5: Cover the vessel with a breathable cloth cover. Set in warm location (ideally 75-85°F [24-29°C])

Step 6: allow to ferment for for 7 to 14 days. Allow to more days you fermente the essential acid to form a Consantrated



Fig.6: fermentation



Fig.9: after fermentation

Secondary fermentation

Step 7: after 14 days add a 15 ml butterfly pea extraction in glass bottle and add a filter the 200ml of kombucha in airtight bottle.

Step 8: Store at room temperature or in the fridge. Wait for 3- 4 days (depending on your personal schedule).

Step 9: drink can be stored for different durations depending on whether it's unopened or opened. Unopened kombucha can last for 1-2 months in the fridge. After opening, refrigerated kombucha will typically last for about a week. At room temperature, kombucha might become too sour to drink within 7-10 days.[33,34]



Fig.10: Secondary fermentation

● Preformulation test

Chemical Identification Tests

Tests specifically for Butterfly Pea (*Clitoria ternatea*), especially targeting its main compounds like anthocyanins and flavonoids.

Standard chemical tests

Chemical Identification of Butterfly Pea Extract

1. Ferric Chloride Test (for Phenolic Compounds)

Purpose: To detect phenolic groups like flavonoids and anthocyanins.

2. Shinoda Test (for Flavonoids)

Purpose: To confirm the presence of flavonoids.

3. Alkaline Reagent Test (for Flavonoids/Anthocyanins)

Purpose: Anthocyanins and flavonoids are sensitive to pH changes.

4. pH Sensitivity Test (for Anthocyanins)

Purpose: To demonstrate Butterfly Pea's pH-dependent color change (basic property of anthocyanins).



5. Lead Acetate Test (for Flavonoids)




Purpose: Another flavonoid confirmation method.


6. Anthocyanin Specific Test (Optional)

Purpose: Specific for detecting anthocyanin pigments.[40,43]

Table 4. chemical Identification Tests

Sr. No.	Test	Procedure	Observation	Image
1.	Ferric Chloride Test	<ul style="list-style-type: none"> Take 2 mL of Butterfly Pea extract. Add 2–3 drops of 5% Ferric Chloride (FeCl_3) solution. 	<ul style="list-style-type: none"> A blue-green, blackish-green, or dark color indicates the presence of phenolics 	
2.	Shinoda Test	<ul style="list-style-type: none"> Take 2 mL of the extract. Add a few small magnesium (Mg) turnings. Add a few drops of concentrated hydrochloric acid (HCl). 	<ul style="list-style-type: none"> A pink, red, or orange coloration appears, confirming flavonoids. 	

3.	Alkaline Reagent Test	<ul style="list-style-type: none"> Add a few drops of sodium hydroxide (NaOH) solution to 2 mL of extract. 	<ul style="list-style-type: none"> A deepening of blue or violet color indicates anthocyanins. Upon acidification (adding dilute HCl), the color shifts to pink. 	
4.	pH Sensitivity Test	<ul style="list-style-type: none"> Prepare two samples of the extract. In one, add a few drops of lemon juice (acid). In the other, add a few drops of baking soda solution (base). 	<ul style="list-style-type: none"> In acidic medium: Blue color turns pink or purple. In alkaline medium: Blue becomes greenish. 	
5.	Lead Acetate Test	<ul style="list-style-type: none"> Add a few drops of 5% Lead Acetate solution to the extract. 	<ul style="list-style-type: none"> A yellow precipitate indicates the presence of flavonoids. 	

6.	Anthocyanin Specific Test	<ul style="list-style-type: none"> • Add 1 mL of extract to 1 mL of concentrated HCl. • Heat the mixture for 5 minutes at 100°C. 	<ul style="list-style-type: none"> • A red coloration indicates anthocyanins are present. 	
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Evaluation test:

• Organoleptic Evaluation

1. Color

Color in butterfly pea kombucha varies from deep blue to pink depending on pH. During fermentation, the acidity increases, changing the color from blue to violet or pink. This visual transformation is due to anthocyanin pigments present in butterfly pea flowers.

Procedure:

Pour 30–50 mL of the kombucha into a clear glass. Place the glass against a white background under natural or white light. observe and describe the color. Record observations using descriptive terms (e.g., deep blue, purple, pink) or a hedonic scale.

2.

Aroma

The aroma of kombucha includes floral and sour notes. Butterfly pea adds a mild, earthy floral fragrance, while the fermentation contributes a slight acidic or vinegary smell.

Procedure:

Swirl the kombucha gently in the glass. Instruct the panelist to sniff the sample 2–3 times without tasting. describe the aroma using terms like floral, sour, mild, or earthy. Record the intensity and pleasantness using descriptive terms or a hedonic scale.

3. Taste

Taste is a combination of sweet, sour, and floral flavors. Properly fermented butterfly pea kombucha has a mild sweetness, balanced acidity, and a subtle floral undertone.

Procedure:

Ask the panelist to sip a small amount of the kombucha. Allow the liquid to roll across the tongue to assess all taste zones. flavors like sweet, sour, tangy, or floral. Use descriptive feedback or a hedonic scale to rate the taste.

4.Mouthfeel

Mouthfeel refers to the physical sensations in the mouth. Kombucha generally feels smooth and lightly carbonated, with a slight astringency in longer fermentations.

Procedure:

focus on texture while sipping the kombucha. Evaluate for sensations such as fizziness, smoothness, or dryness. Describe using terms like light, bubbly, smooth, or slightly dry. Record impressions qualitatively or on a 9-point scale.

5. Overall quality

considering all the sensory attributes together.

Procedure:

Take a sample and observe the kombucha

6.

Appearance

Appearance refers to the overall visual quality of the kombucha, including color uniformity, clarity, presence of sediments, and bubbles or foam. Butterfly pea kombucha is visually attractive due to its unique, natural color (blue to pink) and slight effervescence. Clarity may vary depending on fermentation time and filtration.

Procedure:

Pour 30–50 mL of kombucha into a clean, transparent glass. Place the sample in front of a white background and observe under good lighting. clarity (clear or cloudy), presence of bubbles or sediment. Record the impressions using descriptive terms like clear, hazy, bubbly, vibrant, or dull, or rate them on a 9-point hedonic scale.

7. Clarity

Clarity indicates how clear the kombucha is. It's affected by yeast, SCOBY particles, and filtration. Clearer kombucha is more visually appealing.

Procedure:

Pour kombucha into a clear glass. Place against a white background under good light. Visually assess: Clear, slightly hazy, or cloudy. Optional: Use a turbidity meter for precise measurement

Physical Evaluation

1.

pH

Level

The pH level of kombucha is crucial because it reflects the acidity of the beverage, which impacts both the taste and the safety of the product. Kombucha undergoes fermentation where yeast and bacteria convert sugars into acids, primarily acetic acid. Initially, the pH of kombucha starts around 5-6, and over time, as fermentation progresses, it drops to around 2.5-3.5, making the drink more acidic. This low pH helps prevent the growth of harmful microorganisms, ensuring the kombucha is safe to consume.

Procedure:

Use a calibrated pH meter to measure the pH of the kombucha sample .Rinse the electrode of the pH meter with distilled water before taking the measurement. Insert the pH meter probe into the kombucha sample and ensure it is well immersed. Wait for the reading to stabilize on the pH meter, then record the pH value. Clean the probe with distilled water after each measurement to ensure accuracy and avoid contamination between samples.

2. Total Soluble Solids (TSS)

Total Soluble Solids (TSS), typically measured in °Brix, indicates the amount of dissolved substances in kombucha, primarily sugars. TSS is important as it gives an indication of the sugar content present before and after fermentation. During fermentation, yeast consumes sugars, resulting in a decrease in TSS as sugar is converted into alcohol and organic acids. Monitoring TSS can help assess the progress of fermentation and predict the flavor profile of kombucha (e.g., sweeter kombucha will have higher TSS).

Procedure:

Use a refractometer to measure TSS. Clean the prism of the refractometer using distilled water before measurement. Place a small drop of kombucha onto the refractometer prism and close the lid. Look through the eyepiece of the refractometer or use the digital display to read the °Brix value. Record the °Brix value. This will indicate the TSS and give insight into the sugar content. Higher °Brix values represent more sugar, while lower values indicate more fermentation and sugar consumption.

3. Turbidity

Turbidity refers to the cloudiness or haziness of a liquid caused by suspended particles. In kombucha, turbidity is influenced by the type and amount of tea leaves, the fermentation process, and the presence of microbial cultures or particles. While a slight cloudiness is common, excessive turbidity may indicate issues like contamination or over-fermentation.

Procedure:

To assess turbidity, simply observe the kombucha in a transparent glass against a white background under good lighting. If the kombucha is particularly cloudy, it may indicate a higher microbial load or particulate matter. For a more quantitative measurement, a turbidimeter can be used, which measures the intensity of light scattered by suspended particles. Record the turbidity level in nephelometric turbidity

4. Temperature

Temperature plays a critical role in the fermentation process of kombucha. Ideal fermentation typically occurs between 24°C to 30°C (75°F to 85°F). Temperatures outside this range can slow down fermentation or lead to undesirable microbial growth. Monitoring the temperature is important for both consistency and safety.

Procedure:

To measure the temperature of kombucha, use a digital thermometer or infrared thermometer. Insert the thermometer probe into the liquid (for direct measurement) or point the infrared sensor at the kombucha surface (for non-contact measurement). Record the temperature in °C (Celsius) or °F (Fahrenheit). Regular temperature monitoring during fermentation ensures optimal conditions for fermentation.

5. Carbonation

Carbonation in kombucha is the result of the natural fermentation process, where yeast converts sugars into alcohol and carbon dioxide (CO₂). The level of carbonation varies depending on the length of fermentation and the sealing of the fermentation container. Kombucha is typically mildly carbonated, but carbonation levels can be controlled by adjusting fermentation time or by bottling with added sugar (secondary fermentation).

Procedure:

Visual Inspection: Observe the bubbles rising when kombucha is poured into a glass. This provides a rough idea of carbonation. Carbonation Tester (Pressure Gauge Method): For more precise measurement, a carbonation tester can be used. This involves sealing the kombucha in a bottle and measuring the pressure generated by CO₂ gas. Higher pressure indicates higher carbonation levels. Brix Value: Another indirect method is to measure the TSS (total soluble solids) or °Brix. Higher levels of residual sugars can often correlate with higher carbonation in bottled kombucha, as fermentation continues during secondary fermentation.

Result

Table 5: Organoleptic Evaluation test

Sr.No.	Parameter	Observation
1.	Color	Deep pink
2.	Clarity	Clear to slightly hazy
3.	Aroma	Slightly sour
4.	Taste	Mild floral
5.	Mouthfeel	Smooth, light fizz
6.	Appearance	Attractive
7.	Overall Quality	Refreshing beverage

Table 6: Physical Evaluation

Sr.No.	Parameter	Observation
1.	PH level	7 days = 3.2 14 days = 2.7
2.	Total Soluble Solids (TSS)	Blue to pink
3.	Turbidity	25-60 NTU
4.	Carbonation	Natural carbonation
5.	Temperature (Fermentation)	25-30°C

■ Conclusion

Kombucha with butterfly pea flower presents a unique and health-boosting beverage that [36] combines the probiotic benefits of kombucha with the antioxidant properties of butterfly pea. This fusion not only enhances the visual appeal with its striking blue-to-purple color changes (depending on pH levels) but also adds subtle floral and earthy notes to the flavor profile. [36,37] Overall, this combination is a promising option for health-conscious consumers seeking a visually appealing, flavourful, and functional drink that supports gut health and provides natural antioxidants. [37]

■ Future Prospective :

The development of kombucha with butterfly pea flower presents a promising direction in the functional beverage industry. As consumer preferences shift toward natural, health-promoting, and visually appealing products, this formulation aligns well with emerging trends. Butterfly pea flower enhances kombucha not only with its vivid blue color and pH-sensitive hue shifts but also with added antioxidant, anti-inflammatory, and neuroprotective properties.

The combination offers opportunities for innovation in product differentiation, particularly in wellness-oriented and clean-label markets. With further research on optimizing fermentation conditions, ensuring anthocyanin stability, and validating health claims, this beverage has strong commercial potential. Additionally, its appeal to health-conscious and younger demographics, driven by sensory novelty and perceived benefits, indicates a growing market demand. Future exploration can expand into shelf-life enhancement, functional customization (e.g., nootropic or adaptogenic additives), and large-scale sustainable production, positioning kombucha with butterfly pea as a next-generation health drink.

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- 2.Kombucha: The Ultimate Guide by K. LaValle (2019)
- 3.The Complete Guide to Kombucha by R. Ravensthorpe (2018)