CHAPTER II
LITERATURE REVIEW

2.1 Ingredients Review

2.1.1 Starter Kombucha and SCOBY

Figure 2. 1 scooby and starter kombucha

Kombucha is a fermented beverage made from sweetened tea and a SCOBY (Symbiotic Culture of Bacteria and Yeast), along with a portion of previously fermented tea known as the starter. These two components play a vital role in initiating and controlling the fermentation process, influencing the product's acidity, aroma, and health benefits (Zubaidah et al., 2022).

The SCOBY is a cellulose-rich pellicle that houses beneficial microorganisms, mainly acetic acid bacteria (AAB) such as *Komagataeibacter* and *Acetobacter*, and yeasts like *Saccharomyces* and *Zygosaccharomyces* (Ojo et al., 2023). Yeasts convert sugar into ethanol and CO_2, while bacteria oxidize ethanol into acetic and gluconic acids, producing the drink's characteristic sour flavor and low pH (Içen, 2023). This interaction also increases the bioavailability of polyphenols and antioxidants (Wang et al., 2022).

In product development, maintaining a healthy SCOBY and properly prepared starter are essential for producing stable, safe, and high-quality kombucha. For new formulations such as butterfly pea and lychee kombucha, the SCOBY and starter are key to achieving balanced acidity, stable color, and desirable flavor while enhancing antioxidant potential.

2.1.2 Filtered water

The use of filtered or dechlorinated water is highly recommended in kombucha production because the quality of water plays a crucial role in the success of the fermentation process. According to Coton et al. (2017), the presence of residual chlorine and chloramine in tap water can inhibit the activity of yeast and acetic acid bacteria that drive kombucha fermentation. These chemical disinfectants may cause unbalanced fermentation, reduce microbial activity, and lower the overall physicochemical and sensory quality of the final beverage.

Figure 2. 2 Filtered water

Furthermore, Villarreal-Soto et al. (2018) explain that the physicochemical properties of water, such as mineral content, pH, and the absence of chemical disinfectants, strongly influence the microbial stability, safety, and sensory characteristics of kombucha. Clean water with balanced mineral composition supports a stable microbial ecosystem within the SCOBY (Symbiotic Culture of Bacteria and Yeast), promoting consistent fermentation and flavor development.

In general, filtered water has a neutral pH, typically ranging from 6.5 to 7.5 (U.S. Environmental Protection Agency, 2019; World Health

Organization, 2017). This range is considered ideal, as it neither inhibits microbial growth nor alters the initial balance of the fermentation environment. During the fermentation process, the pH naturally decreases to approximately 3.0–4.2, which is regarded as a safe and desirable condition for mature kombucha (Jayabalan et al., 2014).

Therefore, choosing clean, filtered, and chemical-free water is crucial for maintaining the quality of kombucha. Good water not only supports the growth of beneficial microbes in the SCOBY but also ensures the final product is safe to consume and has optimal flavor.

2.1.3 Butterfly Pea Tea

Figure 2. 3 Butterfly Pea Tea

Butterfly pea flower *(Clitoria ternatea L.)* is a plant rich in bioactive compounds such as anthocyanins, flavonoids, and polyphenols, giving it significant potential as a base ingredient in the production of functional beverages. One method of development is through kombucha fermentation. Research shows that butterfly pea flower-based kombucha has a higher phenol and flavonoid content than a brew of the flowers alone, and has been shown to inhibit pancreatic lipase with an IC50 value of 162.83 mL, indicating its potential as an anti-obesity agent (Biogenesis Journal, 2023).

Furthermore, the fermentation time significantly influences the chemical characteristics and antioxidant activity of butterfly pea flower kombucha. Fermentation for 8 days reportedly produced optimal antioxidant activity of 89.74%, which is considered very high (International Journal of Chemistry & Material Science, 2022). This indicates that the combination of the fermentation process and the bioactive compounds of butterfly pea flower can significantly increase antioxidant capacity.

Furthermore, the addition of butterfly pea flower extract to fermented black tea kombucha has been shown to enrich flavonoid content and provide potential anti-inflammatory activity (DOAJ, 2021). In vivo studies also show that consuming butterfly pea flower kombucha can improve metabolic profiles under high-fat diets, including lowering triglyceride and total cholesterol levels, and improving gut microbiota balance (PubMed, 2022).

Furthermore, butterfly pea flower kombucha also possesses broad-spectrum antibacterial activity. Research reports that at a sugar concentration of 40%, butterfly pea flower kombucha can inhibit the growth of Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, and Escherichia coli (Biotek Journal, 2022). With these findings, butterfly pea flower tea in the form of kombucha not only provides a beverage with an attractive color and distinctive flavor, but also offers a variety of health benefits, ranging from antioxidant, anti-inflammatory, anti-obesity, and antibacterial activity.

Table 2. 1 Nurtitional Value of Butterfly Pea Tea

No	Component	Amount	Functional Benefit
1	Calories	12 kcal	Provides energy
2	Carbohydrate	2.5 g	Main source of energy
3	Protein	0.4 g	Supports cell structure and tissue repair
4	Fat	0.1 g	Minimal content
5	Fiber	0.8 g	Promotes digestive health
6	Anthocyanins	50–60 mg	Acts as a natural antioxidant, combats free radicals, and serves as a natural colorant

(Source: Akshaya et al., 2021; USDA, 2019)

2.1.4 Sugar

Figure 2. 4 sugar

Sugar plays a crucial role as the primary substrate in kombucha fermentation. The yeast in the SCOBY breaks down sucrose into glucose and fructose, then converts it into ethanol and carbon dioxide. This ethanol is then oxidized by acetic acid bacteria into organic acids such as acetic, gluconic, and lactic acids, which impart a distinctive sour flavor and natural preservative properties to kombucha (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). The initial sugar concentration significantly influences the fermentation rate, microbial growth, and the bioactive compound content of the final product (Coton et al., 2017). Therefore, sugar serves not only as a sweetener but also as an essential energy source for the microbes that produce high-quality kombucha.

Table 2. 2 Nutritional Value Of Sugar

No	Component	Amount	Source of Benefit
1	Calories	12 kcal	Energy

	Carbohydrates	300 g	Fermentation substrate
3	Protein	0 g	–
4	Fat	0 g	–

(source : USDA, 2019)

2.1.5 Lychee

Figure 2. 5 Lychee

The use of canned or chopped lychee (Litchi chinensis) as an additive in kombucha production offers both practical and functional advantages. Canned lychee provides readily available soluble sugars for yeast during fermentation—particularly in the secondary fermentation stage—as well as phenolic and aromatic compounds that enrich the sensory profile and antioxidant activity of the final beverage (Jin et al., 2023; MyFoodResearch et al., 2023).

However, the thermal processing involved in canning modifies the chemical composition of the lychee aril. Several studies have reported increased phenolic extraction and antioxidant activity immediately after heat treatment, although degradation of certain compounds may occur during storage. Therefore, the chemical characteristics of lychee syrup (°Brix, pH, and sugar composition) should be considered when designing fermentation parameters to prevent excessive alcohol production and overcarbonation. Moreover, since canned products typically undergo thermal sterilization, the microbial contamination risk from the fruit itself is relatively low. Nonetheless, the high sugar concentration in lychee syrup requires careful adjustment of dosage and fermentation time to maintain the balance between yeast and acetic acid bacteria (Wang et al., 2020; Moreira Terhaag et al., 2025).

Studies on fruit-based kombucha indicate that each fruit type differently influences pH, total phenolic content, and antioxidant capacity. Therefore, the use of canned lychee should be empirically tested within a concentration range (e.g., 5–20% v/v during secondary fermentation or blending prior to the first fermentation), with daily measurements of °Brix and pH, organic acid profiling, and sensory analysis to determine a formulation that achieves an optimal balance between microbiological safety, color and aroma stability, and the functional (antioxidant) value of the final product (Jin et al., 2023; Zubaidah et al., 2023).

Table 2. 3 Nutritional Value Of Lychee (per 100g)

No	Component	Amount	Source of Benefit
1	Calories	66 kcal	Energy
2	Carbohydrates	16.5 g	Energy, natural sweetness
3	Natural Sugars	15 g	Secondary fermentation substrate
4	Protein	0.8 g	Minimal contribution
5	Fat	0.4 g	Minimal contribution
6	Vitamin C	71.5 mg	Antioxidant,immune support
7	Polyphenols	10–15 mg	Antioxidant

(Source: Jiang et al., 2013; USDA, 2019)

2.1.6 Black Tea

Figure 2. 6 Black tea

Black tea *(Camellia sinensis)* is a common base ingredient for kombucha production due to its distinctive phenolic compounds including theaflavins and thearubigins which undergo transformation during fermentation and contribute to the antioxidant activity of the final beverage. Fermentation of kombucha based on black tea alters its physicochemical profile (pH, organic acids) and synthesizes functional metabolites, resulting in increased total phenolic content and antioxidant capacity under certain fermentation conditions; however, these changes strongly depend on fermentation time, sugar concentration, and the composition of the microbial starter (de Noronha et al., 2022; Zhou et al., 2022).

Black tea based kombucha also exhibits different phenolic profiles and bioactive potential compared to kombucha made from other tea types, and several recent studies have reported metabolic effects as well as modulation of gut microbiota following regular consumption, although clinical studies remain limited and require further replication (Kitwetcharoen et al., 2024; Fraiz et al., 2024; de Campos Costa et al., 2025).

For formulation studies, it is recommended to examine various tea concentrations and fermentation durations, while measuring phenolic parameters, antioxidant activity, pH, and microbial composition to gain a comprehensive understanding of how black tea influences the quality and functional potential of kombucha (Wiley et al., 2025).

2.2 Product Review

Butterfly Pea tea and Lychee Kombucha is a natural fermented beverage that combines butterfly pea tea, black tea, and fresh lychee fruit. The two-stage fermentation process produces a vibrant purple-brown beverage rich in probiotics, with a balanced floral–fruity flavor and a slight natural acidity typical of kombucha.

This drink contains a small amount of caffeine derived from black tea, providing a mild stimulating effect, while still maintaining a low-caffeine profile compared to coffee or regular tea beverages.

Butterfly pea flowers contribute anthocyanins, which act as antioxidants and natural pH indicators (Khoo et al., 2017), whereas black tea supplies polyphenols and theaflavins that enhance flavor complexity and antioxidant activity (Jayabalan et al., 2022). Meanwhile, lychee adds vitamin C and a refreshing tropical aroma that complements the floral notes of butterfly pea (Mahattanatawee et al., 2006).

This kombucha is produced without artificial colorants or preservatives and is packaged in eco-friendly glass bottles. With its visually appealing color, digestive health benefits from probiotics (Villarreal-Soto et al., 2018), and its refreshing yet functional taste profile, the product is highly suitable as a healthy fermented beverage made from local natural ingredients.

2.3 Process Review

2.3.1 Boiling

Boiling is an essential preliminary step in the production of butterfly pea and black tea-based kombucha. Boiling is defined as the process of heating a liquid until its vapor pressure equals atmospheric pressure, causing the liquid to transition into vapor (Doretti et al., 2017).

In the context of kombucha fermentation, this step serves to extract bioactive compounds from herbal and tea ingredients while simultaneously sterilizing the brewing solution from potential contaminating microorganisms. At the initial stage of kombucha preparation, water is heated to boiling (~100 °C), after which dried or fresh butterfly pea flowers and black tea leaves are added simultaneously.

The mixture is boiled for 5–10 minutes to extract a wide range of bioactive compounds from both ingredients. From the butterfly pea flowers, anthocyanins, flavonoids, and other phenolic compounds are extracted, which are responsible for the bluish-purple coloration and antioxidant activity of the beverage (Khoo et al., 2017). From black tea, the process releases tannins, catechins, theaflavins, and thearubigins, which contribute to the beverage's characteristic flavor, dark color, and antioxidant as well as antimicrobial properties (Jayabalan et al., 2022).

Beyond extraction, boiling also functions as an initial sterilization step, as high temperatures destroy most vegetative microorganisms present in the raw materials. This helps reduce contamination risks during the fermentation process (Okpala et al., 2019). Once boiling is complete, the tea mixture is filtered to remove plant residues and cooled to room temperature (approximately 25–30 °C) before adding the starter liquid (previous kombucha) and SCOBY (Symbiotic Culture of Bacteria and Yeast).

Several studies have indicated that boiling natural ingredients such as butterfly pea flowers and black tea may influence the stability of their bioactive compounds, particularly anthocyanins and polyphenols. Although some heat-sensitive compounds may degrade, moderate heating for a short duration enhances the solubility and release of active constituents, thus improving the antioxidant activity of the resulting infusion (Sutharut & Sudarat, 2012).

Furthermore, subsequent kombucha fermentation after boiling leads to an increase in total antioxidant capacity, due to microbial metabolism during fermentation that transforms complex polyphenols into simpler, more bioactive forms (Villarreal-Soto et al., 2018). Therefore, the combined boiling of butterfly pea flowers and black tea not only yields a distinctive color and aroma but also supports enhanced antioxidant potential and overall functional value of the resulting kombucha beverage.

2.3.2 Sterilization

Bottle sterilization is a crucial step in kombucha production to ensure that fermentation vessels are free from microorganisms that could disrupt the growth of the SCOBY (Symbiotic Culture of Bacteria and Yeast) or contaminate the final product. As a naturally fermented beverage, kombucha is highly susceptible to contamination by pathogenic microbes and molds if equipment and containers are not properly sterilized.

The most common method of bottle sterilization for kombucha is boiling sterilization. Glass bottles are first washed with soap and hot water to remove dirt and grease, then boiled in water for 15–30 minutes. This method is intended to kill vegetative microorganisms such as bacteria and fungi (Okpala et al., 2019). After boiling, bottles are dried by inverting them on a clean cloth or sterile rack without touching the inner surfaces.

When available, a more effective method is autoclaving at 121 °C under 15 psi pressure for at least 15 minutes, which can destroy even bacterial spores. However, for home or small-scale production, boiling is sufficiently effective if performed properly. It is equally important to sterilize bottle caps and any tools that come into direct contact with kombucha.

Some fermentation practitioners also use food-grade sanitizing solutions (such as peracetic acid or a hot water–vinegar mixture) to rinse bottles prior to use. Bottle sterilization not only ensures food safety but also guarantees that fermentation proceeds optimally and that kombucha’s flavor remains unaffected by external contaminants.

2.3.3 Humidity

Environmental humidity plays an indirect yet significant role in the safety, fermentation stability, and sensory quality of kombucha. The fermentation process is ideally carried out at a temperature of 25–30°C with a relative humidity (RH) of around 60–70% (Zhou et al., 2022; Aksornsri et al., 2023).

At this humidity level, the surface of the medium and the SCOBY (Symbiotic Culture of Bacteria and Yeast) remain moist, ensuring stable microbial metabolic activity. Low humidity levels (<50%) can cause the SCOBY surface to dry out, inhibit the growth of acetic acid bacteria, and reduce organic acid production. Conversely, excessively high humidity (>80%) can lead to water condensation on the lid, dripping back into the medium and increasing the risk of mold contamination (Wahono et al., 2024).

2.3.4 First Fermentation

The first fermentation stage of kombucha using a combination of black tea *(Camellia sinensis)* and butterfly pea flower *(Clitoria ternatea)* serves as the primary phase for the formation of a fermentative substrate rich in reducing sugars, organic acids, and phenolic precursors that are essential for SCOBY activity and the development of the beverage's initial sensory profile (Aksornsri et al., 2023).

During this phase, a rapid pH decrease and the conversion of sucrose into ethanol and organic acids (e.g., acetic acid, glucuronic acid) occur. These processes are strongly influenced by fermentation duration, temperature, and the ratio of black tea to butterfly pea flower; these parameters also determine the stability of anthocyanins from butterfly pea and the transformation of theaflavins/thearubigins from black tea, thereby affecting the final antioxidant capacity of the product (Sintyadewi et al., 2024; Sanwal, 2023).

Several experimental studies have reported that adding butterfly pea to a black tea base can enhance total phenolic content and antioxidant activity during the early to mid-fermentation period (often optimized within 7–10 days), although prolonged fermentation may lead to anthocyanin degradation and undesirable color changes (Wahyanto et al., 2024; Meilani et al., 2021).

From a microbiological perspective, this substrate combination may modulate the dynamics of yeasts and acetic acid bacteria during the early phase—such as accelerating yeast activity due to additional sugars from the flower or fruit extracts—which in turn influences the organic acid profile and functional metabolite formation (Aksornsri et al., 2023; Sanwal, 2023).

Therefore, the design of the first fermentation for black tea + butterfly pea formulations should include variations in substrate ratios, daily pH and Brix/residual sugar measurements, total phenolic/anthocyanin assessments, and microbiota monitoring to determine the optimal fermentation endpoint that balances color, antioxidant activity, and sensory characteristics (Sintyadewi et al., 2024; Wahyanto et al., 2024).

2.3.5 Second Fermentation

Secondary fermentation is a subsequent stage in kombucha production carried out after the completion of primary fermentation. The main purpose of this stage is to enhance flavor, aroma, carbonation (natural bubbles), and enrich the bioactive compound profile of the beverage. In butterfly pea kombucha, the second fermentation is performed by adding fresh lychee fruit to the kombucha that has already undergone 7–10 days of primary fermentation. Lychee not only imparts a distinctive sweet tangy tropical aroma but also contributes vitamin C, phenolic compounds, and flavonoids that can enhance the antioxidant activity of the final product (Mahattanatawee et al., 2006).

After the SCOBY is removed from the kombucha liquid, the fermented tea is strained and poured into clean, sterilized glass bottles. Peeled and chopped lychee (approximately 10–15% of the kombucha volume) is added into each bottle. The bottles are then tightly sealed and stored at room temperature for 2 to 3 days. During this secondary fermentation, yeasts still active in the kombucha ferment the natural sugars of the lychee, producing carbon dioxide (CO_2). Since the bottles are sealed, the CO_2 becomes trapped, resulting in natural carbonation. This process also enriches the flavor and aroma of kombucha, making it more complex and refreshing.

Because this fermentation takes place in sealed conditions, it is important to monitor pressure buildup to avoid bottle explosions caused by excessive gas accumulation. Typically, on days 2–3, bottles can be "burped" by briefly opening them to release excess pressure. After secondary fermentation is complete, kombucha can be filtered again (to remove lychee pieces) and stored in the refrigerator. Cooling halts further fermentation and preserves flavor stability.