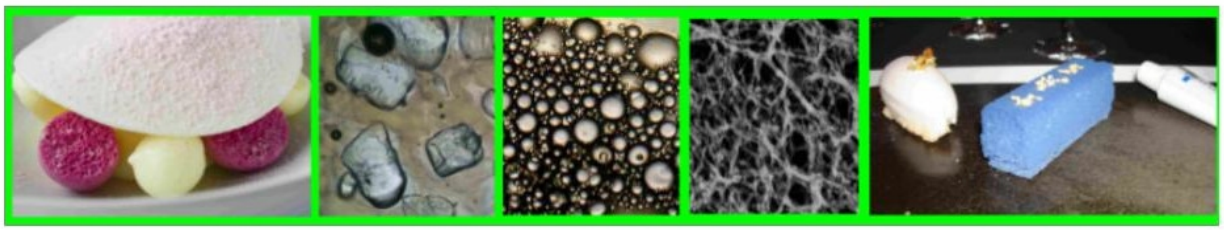

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Kombucha, a complex fermented beverage

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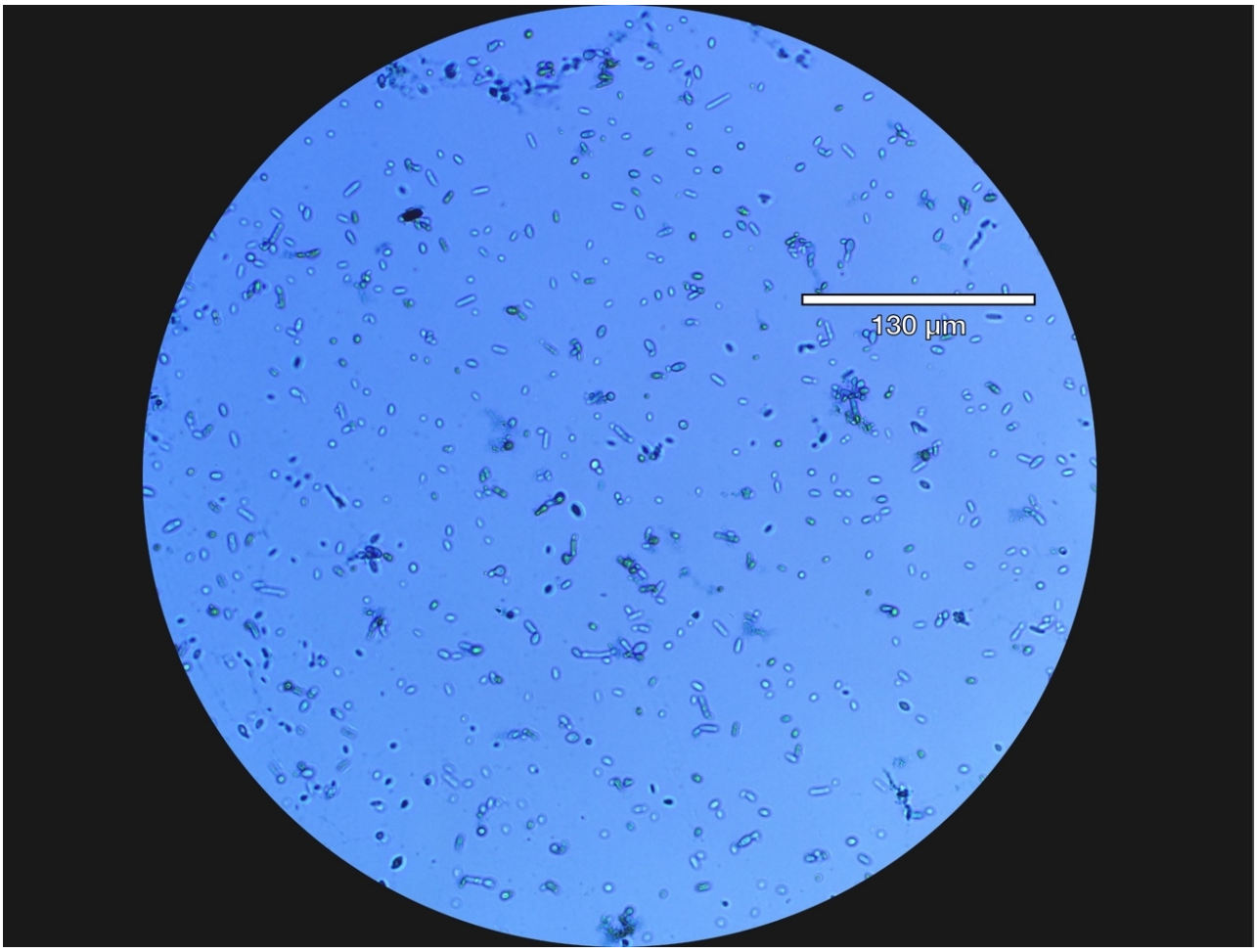

Microscopic image of symbiotic culture of bacteria and yeast (SCOBY) in fermented kombucha showing yeast and bacteria (taken at 400 × total magnification).

Kombucha is a fermented tea that undergoes a fermentation process using a symbiotic culture of bacteria and yeast, known as a SCOBY (Yang *et al.*, 2010). Its origin can be traced back to northeast China's Manchuria region during the Tsin Dynasty around 220 BC (Greenwalt *et al.*, 1998). In the last decade, there has been a growing interest in consuming this fermented tea-based beverage in Western society, particularly within the functional food movement, due to its perceived health benefits (Jayabalan *et al.*, 2014).

The process of making kombucha

Kombucha is prepared by aerobic and static fermenting sucrose-sweetened black, green, or blue (Oolong) tea with a SCOBY (Coton *et al.*, 2017). The primary step in the fermentation of kombucha involves producing a sweetened tea infusion by dissolving 50 -100 g of sucrose per liter of boiling water (Dutta and Paul, 2019). After the sucrose is dissolved, loose tea leaves or tea bags are added and steeped for approximately 5 minutes before being removed. The sweetened tea infusion is then cooled to 20 °C and inoculated with approximately 24 g of SCOBY (Jayabalan *et al.*, 2014) and up to 20 % of a previously fermented batch of kombucha (Toth, 2021). The addition of previously fermented kombucha is referred to as 'backslopping' (Hutkins, 2006). In fact, this practice is utilized in other natural food and beverage fermentation processes, such as the production of sourdough, fermented meats and cereals, and less commonly, water and milk kefir (Laureys and De Vuyst, 2011). Backslopping is used in kombucha production as it contains indigenous yeast and bacteria which can reduce pH during fermentation. The reduction of pH is essential to inhibit the growth of pathogens which include *Clostridium perfringens*, *Bacillus cereus,* and *Clostridium botulinum* (Coton *et al.*, 2017).
Following inoculation, fermentation occurs at ambient temperatures between 18 - 28 °C (Teoh *et al.*, 2004) with the fermentation period ranging from 7 to 60 days (Chu and Chen, 2006), but a period of 8 to 14 days is widely used as it limits acetic acid character (Dutta and Paul, 2019). Commonly, a cloth cover is placed on the fermentation vessel to prevent contaminants and pests from entering. In addition, this also allows the influx of oxygen and prevents pressure build up due to the production of carbon dioxide as a byproduct (Jayabalan *et al.*, 2014). The characteristic sour taste of kombucha is attributed to the presence of acetic, gluconic, tartaric, malic, and citric acids, which are the primary acids produced during fermentation (Jayabalan *et al.*, 2014). However, it is common for the kombucha to undergo carbonation through a secondary fermentation in a tightly sealed vessel. At this stage, various alterations to kombucha can be done to create interesting and unique flavor experiences. One popular method involves adding fruit juices and natural extracts to the kombucha (Laureys *et al.*, 2020).

The microbial ecosystem

Kombucha fermentation is complex due to the diversity of microorganisms involved and their interactions which can stimulate or inhibit each other's growth rate (Markov *et al.*, 2003). While the microbes discussed below have well-known roles in the fermentation process, there are still many other species whose roles and interactions are not fully understood because species diversity varies from fermentation to fermentation (Villarreal-Soto *et al.*, 2018). The dominant bacterial population in kombucha is composed of acetic acid bacteria (AAB: *Komagataeibacter*, *Gluconobacter*, and *Acetobacter* species), which are aerobic bacteria that utilize ethanol as a substrate for acetic acid production (Roos and Vuyst, 2018). During the early stages of fermentation, the yeast species *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* are reportedly dominant. However, as fermentation progresses, acetic acid bacteria, particularly *Acetobacter* and *Gluconobacter* species, became more abundant (Tran *et al.,* 2020). Unlike yeast, these bacteria require an oxygen supply for their growth and metabolic functions (Jayabalan *et al.,* 2014). Lactic acid bacteria (LAB: *Lactobacillus*, *Lactococcus*) are present in

kombucha in low abundance and play a more subtle role in the fermentation process (Marsh *et al.,* 2014). In contrast, yeasts (*Schizosaccharomyces pombe, Saccharomyces ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Torulaspora delbrueckii,* and *Brettanomyces bruxellensis*) play an integral role in the fermentation process. They hydrolyze sucrose into D-glucose and D-fructose, leading to ethanol production (Coton *et al.*, 2017). Following this, AAB transform the ethanol into acetic acid, and the production of D-gluconic and D-glucuronic acids occurs. Higher levels of acetic acid are linked to the abundance of *Acetobacter* species, while increased ethanol levels are correlated with the dominance of yeast species (Tran *et al.*, 2020). These processes result in two final products: the fermented tea and the distinctive microbial cellulose biofilm (pellicle) that resides at the liquid surface. The pellicle is produced through a specific metabolic process utilizing a variety of carbon sources, including D-glucose, ethanol, sucrose, and glycerol (Watawana *et al.*, 2016). *Komagataeibacter* species are thought to be responsible for the production of the pellicle (Semjonovs *et al.*, 2017).

Fermentation factors

Kombucha fermentation is influenced by several factors, such as the microbial population, aeration, temperature, shape, fermentation duration, and size of the fermentation vessel (Dutta and Paul, 2019). Any alterations in these factors can impact the rate of fermentation, the range of compounds produced, the sensory qualities of the end-product, its nutritional value, and other physical and chemical characteristics (Villarreal-Soto *et al.*, 2018). Two of the most important and controllable parameters for kombucha fermentation are the duration of fermentation and temperature. The duration of fermentation significantly impacts the sensory characteristics of the final product with studies indicating that a fruity and refreshing drink can be produced within 6 to 10 days of fermentation, while an extended period results in a vinegar-like taste (Reiss, 1994). Regarding the effect of temperature, if the fermentation process is carried out at the reported optimum temperature range of 22 - 30 °C, it can result in enhanced microbial growth and enzyme activity, leading to better fermentation outcomes (Hur *et al.*, 2014).
Kombucha is a fermented beverage with a complex microbiological composition, furthermore, it is an under-researched fermented beverage. Conducting more research on kombucha is needed to gain a deeper understanding of the microbiological composition and chemical properties that occur during fermentation processes, and thereby assist in optimizing the fermentation conditions and ultimately producing a superior product for a pleasing hedonic experience.

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