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Review

The Technological Perspectives of Kombucha and Its Implications for Production

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Abstract: Fermentation is one of the oldest biotechnological tools employed by mankind. Fermenting food gives them better sensory and nutritional qualities, the latter including vitamins, phenolic compounds, antioxidants, and antimicrobials. Kombucha is the result of the fermentation of a sweetened *Camellia sinensis* infusion by the action of a symbiotic community of yeasts and bacteria organized in a cellulosic biofilm called SCOBY and has gained great prominence among fermented foods and beverages, with a considerable increase in its popularity in the last decade, both among consumers and within the scientific community. This is explained by the particular functional and microbial characteristics of this beverage, such as its antioxidant and antimicrobial potential, long-term stable microbial communities, its suitability for fermentation under different conditions of time and temperature, and amenability to other carbon sources besides sucrose. Thus, this review aims to present and discuss the functional, microbial, and physicochemical aspects of kombucha fermentation, covering the many challenges that arise in its production, in domestic, commercial, and legislation contexts, and the next steps that need to be taken in order to understand this drink and its complex fermentation process.

Keywords: traditional fermented food; heath; food technology; microbiome

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1. Introduction

One of the oldest methods of preserving food is through the fermentation process. The metabolites produced during fermentation generate desired characteristic flavors and decrease food matrix pH, inhibiting the growth of pathogenic bacteria increasing shelf life [1]. Fermented foods and beverages have been part of the cuisine of diverse populations for thousands of years, all over the world, as explored in Sandor Katz' book, *The Art of Fermentation*, an important reference for artisanal producers [2]. Fermented foods were probably the earliest processed foods, and the ancient fermentation techniques were known by philosophers and alchemists [3]. Fermented foods, such as kefir, kombucha, sourdough bread, kimchi, sauerkraut, tempeh, natto, and miso, and others, including, as some of the most popular products, vinegar, beers, wines, and cheeses, are used in our daily lives and represent the oldest known uses of biotechnology [4,5]. Concerning public health and food culture, fermented foods confer microbiological safety and correspond to about a third of the world's diet [5]. It is known that there is a risk that foods of animal and vegetable origin may be contaminated with viruses, fungi, and bacteria, which make disease outbreaks transmitted by common foodstuffs a considerable public health problem worldwide [6]. Thus, this review aims to present and discuss the functional, microbial, physicochemical, and safety aspects of kombucha fermentation under the lens of the differences identified in the literature regarding the home and industrial production of this traditional fermented beverage.

2. Kombucha

The history of fermented foods runs together with the development of civilizations, ensuring cultural aspects and nutritional importance for communities in different countries [3]. In terms of culture, kombucha's worldwide popularity has fluctuated since 1939. It has recently become popular in the United States, arriving in other countries, such as Brazil, together with functional foods. This popularity was motivated by the present interest in the relationship the between microbiome and health benefits, especially as shown by the final consumer, and the growing awareness of food quality [7].

Fermented foods, mainly due to their high concentrations of organic acids and low pHs, as well as essential compounds, such as bacteriocins, preventing the growth of pathogenic bacteria, such as *Helicobacter pylori*, *Escherichia coli*, *Salmonella typhimurium*, and *Campylobacter jejuni*, constitute a safe and relevant choice for nutrition in various circumstances. In addition to containing antimicrobial components, fermented foods play an essential role in improving the social well-being of people living in marginalized and vulnerable societies, providing "safe and protected" status, as pointed out by El Sheikha [3]. One of these fermented beverages is kombucha, a refreshing drink, slightly sweet and a little acidic, usually consumed cold, and which may contain residual carbon dioxide, i.e., is gasified by varying the intensity of the formation of bubbles. It is obtained by the fermentation of black tea or green tea (*Camellia sinensis*) with added sugar and SCOBY (symbiotic culture of bacteria and yeast) [8,9]. During the fermentation, the action of yeasts metabolizes sucrose into fructose and glucose to produce ethanol. This alcohol is oxidized by bacteria, which occurs in the presence of oxygen, resulting in acetic acid [8]. The SCOBY is a biofilm of microorganisms which initiate the fermentation of sweetened tea, occurring usually during 4 to 10 days. After fermentation, kombucha presents sugars, polyphenols from tea, organic acids, fiber, ethanol, amino acids, including lysine; essential elements, such as copper, iron, manganese, nickel, and zinc; water-soluble vitamins. such as vitamin C and various complex B vitamins; carbon dioxide; substances with antibiotic potential; and enzymes [6].

An interesting point to be made about traditional fermented foods and beverages is that they are usually produced in a domestic environment. SCOBY is commonly acquired as a donation, the mother of kombucha, disseminating the culture. Thus, it is difficult to precisely know its origin, though, according to Dufresne and Farnworth [10,11], it was originated in northeastern China (Manchuria), being used during the Tsin Dynasty, around 220 BC, for its detoxifying and energizing properties. It was only in 414 that the physician Kombu took the tea fungus to Japan and used it to cure the emperor's digestive problems [10]. As trade routes expanded, kombucha (former trade name "Tea Kvass") found its way to Russia and, later in the 20th century, to other areas of Eastern Europe, appearing in Germany [10]. Interest in kombucha grew slowly, until mid-2008, when there began an almost exponential growth in the number of media and scientific publications discussing it. However, little is known about this beverage still, especially because of its apparent complex network of microbial interactions and its vast range of metabolic characteristics and a lack of standardization and control over manufacturing processes. The papers referring to kombucha in the literature are very scarce. According to Kim and Adhikari [12], until 2019, there were only 354 publications on kombucha in indexed databases, including conference abstracts, book chapters, scientific papers, and magazine articles, of which only 8 were review papers focusing on kombucha. Thus, little is known about the metabolic, biochemical, and microbial dynamics and correlations of the kombucha manufacturing process, as well as their implications throughout the process and in the final product.

The fermentation process generates bioactive peptides and biogenic amines. It converts phenolic compounds into biologically active ones, as well as reducing the presence of anti-nutrients, culminating in multiple functional properties, such as anti-inflammatory potential, antioxidant activity, lowering of cholesterol levels and blood pressure, reducing the spread of some types of cancer, and improvement of the liver, immune system,

and gastrointestinal functions [4,9,11,13,14]. Among these functional claims, it has also been pointed out that it potentially contains probiotic microorganisms yet to be studied—microorganisms that, when administered in adequate amounts, bring benefits to the health of those who consume them [15,16]. Functional foods are associated with health benefits beyond the basic nutritional properties. Health Canada defines a functional food as a product that resembles traditional food but has demonstrated physiological benefits [17]. In this case, more research is needed to prove the health benefits of kombucha as well as other traditional fermented foods and beverages. In addition, to produce a solid scientific base of knowledge to later apply this data safely, both in preventing and fighting various diseases and disorders in the population, it is essential to understand these benefits [18].

Understanding the physiological benefits of functional foods is essential; however, the risks to human health must also be evaluated. There are biological and chemical risks, so it is necessary to take into consideration the critical control points through the HACCP and ensure good manufacturing practices to obtain safe food of desired quality. Biological risks include the presence of *Clostridium perfringens* and *Bacillus cereus* spores, molds, and pathogenic bacteria due to cross-contamination, especially in kombucha production, which features open fermentation. Chemical hazards derive from the utensils and containers used in the process, as there can be leaching of metals by acid corrosion, resulting in the beverage being contaminated with these metals. There are also dangers associated with excess fermentation, which can increase the amount of acetic acid to levels harmful to health, causing acidosis if a drink with a pH lower than 2.5 is consumed in quantities more significant than 350 mL; such drinks should not be consumed by immunocompromised individuals [8]. Regarding kombucha, in addition to these quality-related factors, it is also important to consider the quantity of the product ingested, eliminating the risk of acidosis mentioned above. The amount of alcohol varies from 0.5% *v/v* to 3% *v/v* depending on the kombucha, so the refrigeration must be well controlled, keeping the product at 4 °C, to significantly reduce the yeast anaerobic fermentation responsible for ethanol production. The concentration of 0.5% *v/v* of ethanol is stipulated in the Brazilian Food Health Affairs [19], which indicates the identity and quality standard of kombucha [7].

In this article, a variety of topics will be addressed concerning the health, sanitary and technological implications of kombucha production. This is, to the knowledge of the authors, the first paper where such topics will be addressed in a systematic review. Initially, the chemical and nutraceutical compositions of kombuchas will be addressed and then their vast, complex, and difficult to standardize network of microbial interactions will be explored. Finally, we will address the different parameters of the fermentation process, such as time, temperature, types of tea and carbon sources, as well as some of their implications for production and future perspectives.

3. Health Benefits

Kombucha has been known about for a long time. However, scientific interest increased at the beginning of the last century. SCOBY metabolites' potential health effects have created a growing interest in kombucha. Due to the efforts of many scientific teams, evidence of the positive effects of kombucha on immune, endocrinological, cardiovascular, gastrointestinal, urogenital, and other levels have been proven in animal models or in vitro. However, no clinical trial analyzing kombucha has been administered to humans. Despite varying concentrations of metabolites and microorganisms, its composition has common denominators among the different formulations present in studies around the world. This topic addresses the health-promoting components of kombucha [20,21].

3.1. Antioxidant Action

Most of kombucha's health benefits are supposedly attributed to its radical scavenging potential. The radical scavenging activity of black tea can be increased through fermentation by the SCOBY microbiota [9]. The antioxidant activity is due to the presence of tea polyphenols, ascorbic acid, vitamin B, and saccharic acid 1,4-lactone (DSL) [6]. The fermentation

promotes structural modification of tea polyphenols and increases the presence of low molecular weight components in the kombucha beverage, contributing a more significant antioxidant activity when compared to unfermented tea [6]. The reason for this difference is linked to variations in the number of infused herbs and antioxidant compounds in black tea and lemongrass [22]. Although kombucha's free radical scavenging properties are directly proportional to the fermentation time, prolonging it is not recommended due to the accumulation of organic acids, which can promote acidosis and may be harmful given direct consumption. Despite the components already noted, identifying extracellular enzymes that act in the structural modification of components during kombucha fermentation and metabolites is necessary to elucidate the active metabolic pathways associated with kombucha's antioxidant effects. Metabolic manipulations can effectively increase kombucha's antioxidant activity and fermentation efficiency [6].

3.2. Detoxification

Some metabolites in kombucha can contribute to the detoxification process. The consumption of glucuronic acid, malic acid, and certain enzymes produced during the kombucha fermentation process, in addition to the interaction between the tea and sugar with the culture of bacteria and yeasts that occurs during the kombucha preparation, can increase the elimination of toxic molecules. This detoxification process is called glucuronidation [23,24].

The uridine glucuronic acid diphosphate (UDP-GlucUA), glucuronic acid bioactive form, can carry detoxifying properties that relieve symptoms of gout, rheumatism, arthritis, and kidney stones [24]. The kidneys can also excrete heavy metals and environmental pollutants after glucuronidation. The membrane transport system uses the highly water-soluble glucuronic acid to transport metabolites through glucuronides products. The glucuronides are excreted products that are absorbed from blood, metabolized, and then secreted into bile or urine. These excreted products are known as glucuronides [10,24]. There has been a strong interest in glucuronic acid in recent years owing to its valuable properties, including its role as a precursor in vitamin C biosynthesis, in addition to its ability to convert to glucosamine and chondroitin sulfate, which are associated with collagen, as well as to the fluid that acts as a lubricant in the joints [24].

The bio-absorbent effect of SCOBY during fermentation also can contribute to the production of a healthier kombucha beverage, considering that heavy metal environmental contaminants, such as arsenic, chromium, and copper, are highly decreased in kombucha [25,26]. Thus, these results reveal that kombucha does not represent potential health risks in terms of toxic element contents [22].

3.3. Probiotic Potential

Kombucha's popularity as a probiotic and symbiotic has increased considerably given its possible functional and probiotic potential. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, provide a health benefit to the host", and these effects must be scientifically demonstrated [15]. Most probiotic bacteria belong to the *Lactobacillus* or *Bifidobacterium* genera [19]. It is known that lactic acid bacteria can exert immunostimulant effects on the host. However, it is not known if the microorganisms present in kombucha can colonize the human gut. Studies with rigorous experimental criteria and controlled microorganism quantities and viabilities should be conducted [15,19]. Humans are home to a complex microbiota consisting of facultative and strict anaerobes, including more than 5000 species. Several reports have indicated that microbial communities in the colon are dominated by five identified phyla: Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Proteobacteria. The question of how symbiotic host–bacteria relationships are maintained without eliciting potentially harmful immune responses is still unresolved [20,27–29]. Probiotics can immunomodulate the local and systemic response.

4. Biodiversity and Population Dynamics in Kombucha

The microbial community in kombucha is an excellent vehicle for transforming the fermentation process and is mainly composed of yeast and acetic bacteria. The microorganisms involved maintain dynamic relationships of cooperation and competition, allowing different ecological succession processes to occur over the fermentation time [30]. In general, the microbial community initiates medium transformation through the action of yeasts, which orchestrate the breakdown of sucrose into glucose and fructose through the action of invertases released in the liquid. As far as these are concerned, such simple carbohydrates will be used as the primary sources of carbon and energy for all microorganisms involved in the community [31]. The preference for the consumption of glucose or fructose is intrinsically related to the species of microorganisms involved, so different studies demonstrate different consumption rates of these carbohydrates in kombuchas of various origins [32,33]. At the beginning of fermentation, the liquid starter contains dissolved oxygen, which will favor the respiratory activity of the yeasts and allow for the initial population growth of yeasts and acetic bacteria. Quickly, the available oxygen decreases, and carbonic acid accumulation will generate some yeast and bacterial ecological niches in the fermenter such that they are able to maintain their metabolic activities in anaerobiosis. Microorganisms, as acetic bacteria, will be present on the surface of the liquid and will form a new cellulosic biofilm (SCOBY), although some acetic bacteria present motility [34].

At first, the fermentation of kombucha involves microorganisms predominantly resistant to the osmotic potential of the medium, given the high concentration of carbohydrates that can reach more than 10° Brix. As these carbohydrates are consumed, the ethanol and organic acid concentrations increase. Ethanol values may vary from culture to culture. However, it will be oxidized by the action of acetic bacteria to produce acetic acid [11,34]. Finally, the liquid beverage becomes dominated mainly by microorganisms that are better adapted to the low pH of the drink, which can usually range from 2.5 to 3.5 [11].

Among the bacteria present in kombucha, acetic bacteria stand out, mainly belonging to the genera *Komagataeibacter*, *Gluconobacter*, and *Gluconacetobacter*, especially cellulose-forming bacteria. These are responsible for forming the cellulosic matrix necessary for SCOBY formation [11,35]. The metabolites involved in kombucha fermentation generate several carbon sources, such as ethanol, glycerol and sucrose, which highly promote the extracellular cellulose fibrils attached to bacteria [11]. Different species can be found in other works in the scientific literature, the most common being *Komagataeibacter xylinus* (Table 1).

Table 1. Some genera and species of bacteria found in kombuchas.

Microorganism	Reference
Komagataeibacter, *Gluconobacter*, *Lyngbya*, *Bifidobacterium*, *Collinsella*, *Enterobacter*, *Weissella*, *Lactobacillus*	[30] (Chakravorty et al., 2016)
Komagataeibacter (*Komagataeibacter rhaeticus*, *Komagataeibacter xylinus*, *Komagataeibacter europaeus*, *Komagataeibacter intermedius*), *Gluconacetobacter*, *Gluconobacter* (*Gluconobacter oxydans*), *Acetobacter* (*A. malorum*, *A. pasteurianus*, *A. pomorum*, *A. tropicalis*)	[36] (Villarrealsoto et al., 2020b)
Acetobacter (*A. xylinum*), *Gluconacetobacter* (*G. xylinus sin. Komagataeibacter xylinus*), *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Bifidobacterium*, *Thermus*, *Allobaculum*, *Propionibacterium*, *Enterococcus*	[16] (Marsh et al., 2014)

In the case of yeast, observed biodiversity is higher than that of bacteria. Adaptive mechanisms present in yeast cover various metabolic maneuvers. Many yeast species present central environmental or stress responses, consisting of gene modulation and activation of stress tolerance mechanisms [36]. These mechanisms involve polyploidy induction, multiplication of large fragments in chromosomes, loci variation in families of Ty1 retrotransposons [37–40]. Such evolutionary maneuvers may have enabled the high diversity of yeasts to adapt to kombucha fermentation conditions (Table 2).

Table 2. Some genera and species of yeast found in kombuchas.

Microorganism	Reference
Candida (*C. stellimalicola*, *C. tropicalis*, *C. parapsilopsis*), *Lachancea* (*L. thermotolerans*, *L. fermentati*, *L. kluyveri*), *Kluyveromyces* (*K. marxianus*), *Debaryomyces* (*D. hansenii*), *Pichia* (*P. mexicana*), *Waitea*, *Eremothecium* (*E. cymbalarie*, *E. ashbyii*) *Meyerozyma* (*M. caribbica*, *M. guilliermondii*), *Zygowilliopsis* (*Z californica*) *Saccharomyces* (*S. cerevisiae*), *Saccharomycopsis* (*S. fibuligera*), *Hanseniaspora* (*H. uvarum*, *H. meyeri*, *H. vineae*), *Kazachstania* (*K. telluris*, *K. exigua*), *Starmera*, *Merimbla*, *Sporopachydermia*, *Sugiyamaella*	[30] (Chakravorty et al., 2016)
Zygosaccharomyces bailii, *Schizosaccharomyces pombe*, *Torulospora delbreuckii*, *Rhodotorula mucilaginosa*, *Brettanomyces bruxellensis*, *Candida stellata*	[41] (Teoh; Heard; Cox, 2004)
Dekkera, *Zygosaccharomyces*, *Kazachstania*, *Davidiella*, *Pichia*, *Wallemia*, *Lachancea*, *Leucosporidiella*, *Kluyveromyces*, *Naumovozyma*, *Meyerozyma*, *Saccharomyces*, *Hanseniaspora*	[16] (Marsh et al., 2014)
Candida arabinofermentans, *Brettanomyces bruxellensis*, *Schizosaccharomyces pombe*, *Zygosaccharomyces bailii*	[36] (Villarrealsoto et al., 2020b)

5. Fermentation Parameters

5.1. Fermentation Colony

Even though there are similarities in the qualitative and quantitative composition of SCOBYs from the kombuchas studied to date [16,41,42], the impact of fermentation colony variation on chemical composition is evident in a comparison of the production kinetics of primary metabolites from nine different kombucha samples in a long period of fermentation, with ethanol concentrations ranging from 0.4% (*v/v*) to 0.7% (*v/v*), which could be explained by differences in the cell counts of yeast and acetic acid bacteria in each sample which lead to specific ethanol production and consumption rates [43]. Villarreal-Soto et al. [44] analyzed the microbial populations of three different SCOBYs through metagenomics methods and predictive metabolomic characterization of metabolic pathways, revealing a strong dependence of kombucha microbiome composition on fermentation kinetics, in addition to the characteristics of the potential secondary metabolites produced in each colony given the set of genes in the gene pool.

5.2. Carbon Source

In the form of prevalent sugar, sucrose is the most common carbon source used to produce kombucha. However, some studies have evaluated other carbon sources, such as molasses, obtaining lower production levels of acetic acid and higher concentrations of lactic acid [32,44,45]. From a commercial point of view, Brazilian legislation restricts kombucha production to the use of the following sugars: sugar cane sucrose, sugar beet, sugar for confectionery, molasses, brown sugar, sweetener, and inverted sugar [46]. Some authors evaluated kombucha fermentation using carbon sources, such as sour cherry juice, grape juice, pomegranate juice, soy milk, and even Jerusalem artichoke extract (*Helianthus tuberosus*). Promising results were found regarding obtaining metabolites of interest, such as glucuronic acid, increased antioxidant potential, or even low-calorie beverages [47–51]. In quantitative terms, Lonc˘ar and colleagues [52] showed that the kinetics of sucrose consumption is complex. Under the experimental conditions proposed in the study, initial sucrose concentrations between 35 g/L and 55 g/L showed the highest reaction rates.

5.3. Tea

The most commonly used vegetable infusion in kombucha studies is black tea, but the use of green tea has been gaining more space in commercial productions, possibly due to its mild flavor and lower price [34]. The amounts used are close to 5 g/L, with slight variations depending on specific recipes to obtain greater or lesser intrinsic properties of tea, such as the number of antioxidants [53,54]. In terms of fermentation kinetics and chemical composition, Kaewkod et al. [55] found significant differences in the parameters

of pH, total acidity, total soluble solids, and organic acid content when compared to green, black, and oolong tea fermentation. Black tea showed a higher rate of total acid production, probably as a result of some effect in the microbial metabolism due to the specific chemical composition of black tea, as the great difference in total acid composition is due to the gluconic acid content, which is formed by the action of acetic acid bacteria upon glucose. Kaewkod et al. [55] also found that the total counts of acetic acid bacteria were significantly higher in green and black tea preparations than in an oolong preparation. Current Brazilian legislation standardizes kombucha as the fermentation of the infusion of *Camellia sinensis*. However, there is no restriction on using other adjuvant plant extracts, as long as they are previously approved as food ingredients [19].

5.4. Amount of SCOBY and Liquid Inoculum (Starter)

A common practice in kombucha production involves adding the SCOBY and some of the fermented liquid produced in the previous batch to the sweetened tea. As acetic bacteria produce cellulose, the bacteria and other microorganisms are partially immobilized in the biofilm structure [11]. Thus, larger amounts of the biofilm imply a more significant contribution of microorganisms. There are studies on fermentation without the presence of SCOBY, with the assistance of microorganisms from previous fermentation processes [32,44,45,51,52]. The commercial manufacturers adopt a significant control of the process, and the previous batch inoculum is often mentioned as decreasing the pH below 4.2 to prevent the proliferation of pathogens. However, it has microbiological activity capable of starting the fermentation process. Lončar and colleagues [52] did not find significant differences between the use of 10% or 15% of inoculum to the total volume and its impact on sucrose fermentation kinetics. Thus, using larger amounts of liquid inoculum can carry excessive acidity to the new fermentation batch, harming the final product, without gains in productivity over time. Furthermore, smaller amounts of liquid inoculum mean a higher yield of batches. The fermentation of kombucha works on a backslopping system.

5.5. Temperature

Considering the varied microbiological composition between yeasts and bacteria and that each microorganism presents a particular response to temperature variation, conducting kombucha fermentation at different temperatures can produce significant differences in the chemical composition of kombuchas produced [54,56,57]. One of the initial steps in the fermentation process of kombucha is the hydrolysis of sucrose into glucose and fructose, which microorganisms will metabolize. This reaction is catalyzed mainly by enzymes present in yeasts and is sensitive to the temperature of the medium. However, there is no simple relationship between sucrose consumption and temperature increase, although this relationship shows a directly proportional effect [52]. Another important factor related to the impact of temperature on fermentation is the possibility of a qualitative change in the microbiological composition and the expected quantitative change. It can beneficially impact the production of some metabolites of interest, as in the case of glucuronic acid, by varying the level of acetic bacteria species. De Filippis et al. [53] showed that the same kombucha can turn from having a predominant population of *Gluconacetobacter xylinus* to a predominant population of *Gluconacetobacter saccharivorans* when the fermentation temperature is shifted from 20 °C to 30 °C, causing a significant change in the glucuronic and gluconic acid contents in the kombucha produced.

5.6. Time of Fermentation

The time of fermentation in studies of kombucha in general ranges from 10 to 14 days [32,44,53,56,57]. Since there is a positive and negative variation of metabolites during kombucha fermentation, the choice of time depends on the desired composition, the desired sensory profile, and a limitation on both acceptance and consumption safety. In legal terms, MAPA in Brazil [19] and the FDA in the USA regulates that pH in kombucha should not be beyond 2.5. It can be noted that, usually, during kombucha fermentation, a

buffer system is established due to the presence of weaky organic acids; this is especially important, as total acidity increased with time for all studied kombuchas even with no remarkable pH changes observed. Total acidity variation in time is, therefore, also a key parameter to define the end of fermentation.

6. Challenges and Technological Aspects in Kombucha Production

A standardization of the physicochemical characteristics of kombucha is required, given the Brazilian Normative Instruction of the Ministry of Agriculture, Livestock and Supply, No. 41, of 17 September 2019. This instruction indicates the following standards of identity and quality for kombucha: (1) alcoholic content between 0.5–1.5% *v/v* for alcoholic beverage ratings; (2) production with drinking water, plant species used as an infusion, sugars, and SCOBY; (3) food additives and technology aids authorized in specific legislation by ANVISA are allowed, in addition to juices, fruit pulp, plant extract, spices, honey, and natural aromatics [19]. Consequently, a standardization of physicochemical characteristics is necessary, as these directly impact the consumer's sensory experience. As it is a beverage fermented through a consortium of microorganisms, some variables must be considered in its production, such as the fermentation kinetics and the defined physical and microbiological properties conferred on the starter culture and final product.

The type of sugar used as a carbon source directly influences the fermentation kinetics of kombucha. The use of pure sucrose provides a lower pH and higher acetic acid content when compared to molasses, while a higher consumption rate of sugar contained in molasses induces a higher lactic acid content [45]. Depending on the carbon source inserted in the process, it is necessary to adjust the fermentation time and temperature, preventing the excessive accumulation of acetic acid and loss of organoleptic properties. In the case of molasses, despite being considerably richer in nutrients than pure sucrose, their metabolites increase pH levels and, what can be consequential, yield a lower quality final product [32]. The complex task of balancing the suitable carbon source with the best efficiency of its metabolism interferes not only with the sensory aspect of the product but also with its safety, since the presence of residual sugar in the packaging of the finished product provides continuous fermentation if not pasteurized, thus increasing the production of gases and alcohol during shelf life, storage, or distribution of the product, affecting the quality of the final beverage. The effectiveness of the distribution system also directly influences this aspect.

Traditional kombucha substrates, such as green or black tea (*Camellia sinensis*), have similar behaviors in fermentation. However, they differ in terms of their physicochemical properties and bioactive compounds profiles [58]. Ahmed and colleagues [59] also used barley or rice as a substrate, reaching a pH of 2.81 and 3.14, respectively, after 10 days of fermentation. This same study also evaluated the acetic acid levels, reaching values of 0.42 g/L and 0.34 g/L after 12 days for barley and rice, respectively, in addition to the alcohol content, with 0.56 g/L and 0.49 g/L, when compared with traditional tea—all known parameters above the desired or recommended levels. There is also an evaluation of the infusion of Yarrow flowers (*Achillea millefolium*) and of subcritical water extracts for fermentation using SCOBY, resulting in higher antioxidant potential, sensory score, and high antimicrobial and cancer cell antiproliferative activity [60]. Other types of information have been tested, such as blends that associate black tea with a specific functional food. The addition of wheatgrass (*Triticum aestivum*) to fermented black tea provided superior and more stable antioxidant activities than traditional kombucha and was recommended for consumption as a new beverage [61].

Kombuchas from different origins also have other antioxidant activities and polyphenol concentrations due to differences in starter culture microbiome, temperature, and time used between fermentations [62]. The stability of polyphenol content during fermentation was first verified in green and black tea residues, in addition to the production of acetic and glucuronic acid, reaching the maximum levels after 15 days for green tea and after 12 days for black tea [63]. It is essential to highlight that, even though the ability to scavenge

free radicals is time-dependent, a prolonged fermentation can accumulate acids at levels potentially harmful to health, which might cause metabolic acidosis [64].

Another factor capable of decreasing or increasing the availability of bioactive compounds is the temperature during the fermentation process, enabling changes in the conditions of the environment and, consequently, selecting certain groups of microorganisms. *Glucanoacetobacteria* species are examples of how temperature interferes in the predominance of their subspecies, which can modify the profile and concentration of organic acids during beverage production, conferring potential beneficial effects on consumer health and modifying sensory characteristics [53]. Identifying which strains are favored in specific temperature ranges is an innovative strategy, aiming to explore the potential of the starter culture and the food matrix (teas) for more significant beneficial effects of the beverage. In this context, one must know the specific characteristics of the SCOBY that is being worked on, since differences in origin present differences in yeast and bacteria species.

At industrial levels, ensuring the permanent impact of a particular strain in the starter culture after successive batches is a challenge, considering that SCOBY is a dynamic symbiotic consortium with microbial proportion variability, despite the stability of the microbial community as a whole. This fact makes microbial diversity one of the most complex factors to control in the standardization of kombucha fermentation. On the other hand, there is a growing need to obtain information on the ways in which the substrates involved in the fermentation process are accompanied by changes in various parameters, such as pH, alcohol, Brix, and, consequently, the organoleptic properties of the final product. Fermentation parameters can be chosen and tested according to the desired end product, whether it is a commercial kombucha with a lower ethanol content or even the evaluation of different substrates from a perspective of alternative carbon sources and production of metabolites of interest. Apparently, the composition of the fermentation colony, temperature, and fermentation time are the most important parameters in the design of a beverage with a chemical composition appropriate to the market and that respects the current legislation.

In this way, it is concluded that we already have a considerable amount of information available to move to the next phase in studying kombucha. We already know the fermentation core of kombucha and the roles of yeast and acetic bacteria in the course of fermentation. We also already know the correlations between time and temperature and how they influence the process and results of fermentation. Given this, certain further studies are necessary, mainly, studies that take into account experiments on a semi-industrial and industrial scale, assuming the possibility of adding different adjuncts and carbon sources. Thus, in the future, approaches on three different fronts of study are desired: (a) omics analysis, taking into account the metabolic and genomic dynamics in a broad way, aiming to identify new points of importance in the kombucha fermentation core, in addition to the dispositions between yeasts and acetic bacteria; (b) studies on the physicochemical variables of kombucha production on a large scale, analyzing differences between fermentation processes, such as comparing single batch, fed batch, and continuous fermentation, as well as investigating the spatial effect of fermenter design disposition, since kombucha fermentation requires an oxygen phase, so the surface area of air–liquid contact can impact fermentation, as can the height of the liquid column; and, (c) last but not least, studies of the real beneficial effects caused by the consumption of kombucha and how these effects may or may not be conserved and presented by the range of microbiomes that different kombuchas present all over the world, presenting themselves as core benefits.

Finally, due to the great difficulty presented in standardizing the fermentation of different kombuchas and given the wide variety of factors and species present and the lack of a scientific consensus, it may be necessary to observe kombucha not as a single product but as a category of fermented beverages. Kombucha may need to be seen as a microecological phenomenon caused by the union of yeasts and acetic bacteria in symbiosis, opening space for new product subclassifications. This fact needs further investigation, and, therefore, the investigative approaches proposed above are necessary, aiming at the

identification of patterns that will allow us to categorize different groups of kombucha within the broad spectrum of fermentation factors.

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