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REVIEW ARTICLE

Kombucha Tea Fermentation: A Review

David Laureys[a], Scott J. Britton[b,c], and Jessika De Clippeleer[a,d]

[a]Innovation centre for Brewing & Fermentation, Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium; [b]Department of Research & Development, Brewery Duvel Moortgat, Puurs-Sint-Amands, Belgium; [c]International Centre for Brewing and Distilling, Institute of Biological Chemistry, Biophysics and Bioengineering, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, UK; [d]Innovation centre for Brewing & Fermentation, School of Bioscience and Industrial Technology, HOGENT University of Applied Sciences and Arts, Ghent, Belgium

ABSTRACT
Kombucha tea is made by aerobically fermenting a sweetened tea infusion with a kombucha culture, a symbiotic culture of bacteria and yeasts. The resulting beverage is usually non-alcoholic, sour and refreshing, but not naturally sparkling. Many consumers are drinking kombucha because of its alleged health effects and as kombucha tea is increasing in popularity, consumers are increasingly demanding kombucha beverages of better quality. To better adapt their products to these consumer demands, brewers are looking to the scientific knowledge about kombucha for answers. However, the scientific exploration of this complex naturally fermented beverage is still limited. This review paper presents the current state-of-the-art. To further deepen the understanding of the kombucha fermentation process and to help brewers in their search for higher-quality beverages, the microbial species diversity and its dynamics during the fermentation process should be investigated in detail, as well as the kinetics of the substrate consumption and metabolite production, and the relationship between the substrate and metabolite concentrations, and the consumer preferences for the resulting beverage.

KEYWORDS
Kombucha; tea fungus; microbial ecology; acetic acid bacteria; yeast

Introduction

Kombucha is a sweetened tea fermented with a symbiotic culture of yeasts and bacteria, typically creating a floating zoogleal mass of microbial cellulose.[1–5] This traditional fermented beverage is thought to originate in northeast China, and was originally valued during the Tsin Dynasty in Manchuria (c. 220 B.C.) for its purported health-promoting properties.[1,4,6,7] As trade routes began to enlarge, kombucha spread to other countries and regions under a variety of different names, including Cainii grib, Fungus japonicus, Cembuya orientalist, Tschambucco, Volga spring, Mo-Gu, Champignon de longue vie, Teekwass, Kwassan, Kargasok tea, Cainii kvass, Japonskigrib, Kambuha, Tea Kvass, Jsakvasska, Heldenpilz, and Kombuchaschwamm.[4,8,9]

Recently, this tea-based fermented beverage has become increasingly popular in western cultures, mainly in the functional food movement for its alleged health benefits. Kombucha tea is thought to reduce cholesterol levels and blood pressure; increase weight-loss; improve liver, glandular, immune, and gastric functions; reduce kidney calcification; increase vitality; combat acne; eliminate wrinkles; purify the gall bladder; improve constipation; alleviate arthritis pain; inhibit cancer proliferation; cure AIDS; and many others.[4,7,10–15] Most of these health benefits are unsubstantiated and based on personal observations and testimonies, but there are some indications that kombucha tea consumption may indeed aid health prophylaxis and recovery through detoxification, antioxidation, energizing, and immune-stimulating effects.[16] However, despite the lack of evidence from clinical trials to substantiate benefits to human health, kombucha is one of the fastest-growing beverages today within the functional food category, demonstrating a + 49% dollar growth over the period of July 2017 to July 2018.[12,15,17]

Kombucha is usually prepared through the aerobic and static fermentation of sucrose-sweetened black, green, or blue (Oolong) tea with a **s**ymbiotic **c**ulture **o**f **b**acteria and **y**east (SCOBY).[7–9,18,19] The preparation usually starts with the dissolution of 50 – 100 g of sucrose per liter of boiling water.[4,14,20,21] Subsequently, tea leaves, either loose or within tea bags, are steeped for a short period, after which they are removed.[4,9] Jayabalan and colleagues[4] reported an addition of 5 g/L tea and a steeping regime of 5 min, but the quantity of tea and steeping time can differ greatly between commercial producers. Thereafter, the sweetened-tea infusion is cooled to 20 °C and inoculated with a part of the SCOBY zoogleal mat and/or part of the previously fermented tea (usually around 20%), which contain large amounts of indigenous yeast and bacteria.[7] The addition of previously fermented tea lowers the starting pH, which may inhibit the growth of human pathogens such as *Clostridium perfringens*, *Bacillus cereus*, and *Clostridium botulinum*, as these microorganisms do not grow in an environment below

CONTACT Jessika De Clippeleer Jessika.DeClippeleer@UGent.be

pH 4.7.[11,22] The antimicrobial effects of kombucha could mainly be attributed to acetic acid.[1] The fermentation is performed under aerobic conditions and at an ambient temperature between 18 – 28 °C, for a period of 8 to 14 days.[6,8,9,23,24] Usually, the fermentation vessel is covered with cloth to keep out fruit flies and other pests.[4] This also prevents pressure build-up in the vessel due to carbon dioxide production while allowing the influx of oxygen. The duration of the fermentation is largely dependent upon the composition and abundance of the microbial population, aeration, temperature, fermentation vessel shape and size, and many other factors.

In the search for novel experiences for the end-consumer, many variations on kombucha have been developed. A common practice is the addition of fruit juices and natural extracts to create novel taste experiences. Recently "hard kombuchas" were developed, which contain 3-11% (v/v) alcohol instead of the 0-1% (v/v) in traditional kombucha.

Microbial composition

Kombucha tea fermentation can be considered a natural fermentation, as its fermentation process is started by adding a certain amount of a previous kombucha fermentation.[4,14,25,26] This practice is referred to as backslopping, which is also used for the production of many other natural food fermentation processes such as sourdough, fermented meats and cereals, water kefir, and milk kefir.[27,28] This practice selects for those microorganisms that thrive under the specific conditions of a kombucha fermentation. As kombucha fermentation is typically performed at the household level with a large variety of ingredients and recipes, the diversity of microorganisms recovered from kombucha is very wide.[4,7,11,14,24,29–34] The most characteristic microorganisms in kombucha fermentations are acetic acid bacteria and yeasts. Lactic acid bacteria can occur, but do not seem to be an essential part of the kombucha microbial ecosystem as they are not always found. Filamentous fungi (molds) can also occur sometimes, but are considered spoilage microorganisms, as they are associated with mycotoxins and adverse health effects.[35]

The kombucha fermentation process typically lasts for 8-14 days at room temperature during which the properties of the sweetened tea change extensively.[33,36] At the start, the medium is aerobic, high in sucrose, and slightly acidic due to the addition of the inoculum. Gradually, the oxygenation of the liquor decreases because of the development of a cellulose layer on top of the fermenting liquor and the consumption of oxygen by the microorganisms in the zoogleal mat and the liquor (AAB and/or yeasts). The growth and metabolism of yeasts, acetic acid bacteria (AAB), and lactic acid bacteria (LAB) result in the accumulation of organic acids and thus decreasing pH values.[4,37] The substrate concentrations gradually decline due to the metabolism of the microorganisms and the production of the cellulose layer. Towards the end of the fermentation process, characterized by a well-developed cellulose pellicle on top of the liquor, and high numbers of yeasts and acetic acid bacteria, a more anaerobic environment might arise with low substrate concentrations and a high acidity. The relatively long fermentation process likely allows for a succession of different microorganisms, as can be derived from previous results.[31] This implies that the end product of this natural fermentation may contain microorganisms that are not optimally adapted to the start of the fermentation process, and vice versa. Designing a more controllable kombucha fermentation process requires a thorough understanding of the wide diversity of microorganisms involved, their role, and their possible succession. Knowing which microorganisms are active at what time during the fermentation process and what metabolic functions they perform is crucial to construct a "designer kombucha" that produces a desired end product. However, the presence and/or dominance of certain microorganisms during natural kombucha fermentations does not imply that they are also the most optimal microorganisms for the desired end product.

Yeast

Yeasts are an integral part of kombucha as they are always present during the fermentation process. A wide diversity has already been recovered, comprising species of the genera *Saccharomyces*, *Zygosaccharomyces*, *Dekkera/Brettanomyces*, *Pichia*, and others.[11,29,38] No specific yeast species was consistently found that characterizes the kombucha fermentation process, but *Dekkera* species might be particularly adapted to the environment.[34,39]

Yeasts are eukaryotic microorganisms belonging to the fungi.[40,41] They are non-motile, around 8 µm in diameter, with spherical or oval-shaped cells. They are facultatively anaerobic but require oxygen for certain growth-maintaining hydroxylations (such as the biosynthesis of ergosterol). Optimal growth is around 20-30 °C and pH 4.5-7.0, but many yeasts can still grow at pH 2.5. Yeasts are not typically associated with negative health effects, although some yeasts are able to produce biogenic amines. Some yeasts can even provide health benefits, as was found for *Saccharomyces cerevisiae* var. *boulardii*.[42]

Yeasts are known for their production of ethanol and carbon dioxide.[43,44] Some yeasts are obligately fermentative and can only perform fermentation (such as *Rhodotorula glutinis*), whereas others are obligately respirative (such as *Kluyveromyces lactis* and *Yarrowia lipolytica*) and can only perform respiration. Most yeasts (such as *Saccharomyces cerevisiae* and *Dekkera bruxellensis*) are facultatively fermentative and can perform fermentation or respiration for the production of energy. Respiration is only possible in the presence of oxygen, but the presence of oxygen does not guarantee a respiratory metabolism.[45] Many yeasts continue to perform fermentation under aerobic conditions as long as carbohydrates are available (often referred to as the "Crabtree effect"). This constitutes part of the make-accumulate-consume strategy to rapidly dominate an ecosystem, outcompete other microorganisms, and stay dominant in that ecosystem. Indeed, when the carbohydrates are consumed, ethanol can be metabolized into carbon dioxide and water. Yeasts can also produce glycerol as a response to high osmotic pressure and for internal redox balancing.[46] Another compound that can be produced by yeasts to maintain a suitable internal redox balance is acetic acid, which is known to result in a decreasing pH during alcoholic fermentation by yeasts.[47,48] This process is also accompanied by the production of higher alcohols and esters, which contribute to the aroma of alcoholic beverages. Higher alcohols are produced by the degradation of amino acids via the Ehrlich pathway, but can also be produced anabolically from pyruvate.[49,50] The medium-chain fatty acids constituting the esters are derived from the fatty acid anabolism for the production of membrane compounds.

When yeasts die, the cells can start a self-degradation process (autolysis), whereby intracellular compounds (such as nucleic acids, proteins, lipids, and polysaccharides) are degraded by endogenous enzymes. This process occurs during the maturation of beer and wine, and results in a wide diversity of compounds, which may affect the flavor of the end products.[51,52] The released nutrients may serve as nutrients for the growth of other microorganisms, such as AAB or LAB.

Acetic acid bacteria

Acetic acid bacteria are the most characteristic microorganisms of kombucha fermentations. Although there are 17 genera of AAB, those found in kombucha belong to the genera *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, and *Komagataeibacter*.[53] In particular, *Komagataeibacter xylinus* is thought to be the most characteristic microorganism of kombucha fermentation, and thought to be responsible for the production of the cellulose pellicle.[4,34] However, in the literature this is not always clear as this microorganism has been classified as *Acetobacter xylinus*, *Acetobacter xylinum*, *Acetobacter aceti* subsp. *xylinus*, *Gluconobacter xylinus*, *Gluconacetobacter xylinus*, and even some additional names.[54,55] Furthermore, the identification of AAB to the species level is often unreliable as phenotypic and 16S rRNA methods are usually insufficient for a reliable identification of AAB. The most characteristic microorganism in kombucha appears to be a cellulose-producing AAB, probably a *Komagataeibacter* species, and maybe a *Komagataeibacter xylinus* strain.[56] *Komagataeibacter* species can accumulate up to 10-20% acetic acid in the medium, whereas *Acetobacter* species can only accumulate a maximum of 8% acetic acid.[57]

AAB are gram-negative (or gram-variable) bacteria belonging to the class α-Proteobacteria and the family of the *Acetobacteraceae*.[58,59] They can be motile, are around 0.5 µm wide and 1-4 µm long, with ellipsoidal to rod-shaped cells, and do not sporulate. Although they are obligately aerobic, they can survive for extended periods under low-oxygen conditions, as is the case in bottled wine and during the cocoa bean fermentation process.[60,61] These low-oxygen conditions may result in a **v**iable **b**ut **n**on-**c**ulturable (VBNC) state, which decreases their recovery via culture-dependent methods.[62] Their optimal growth is ~25-30 °C and ~ pH 5.0-6.5, but many also grow at pH 3.0-4.0 and even lower. AAB are generally not considered to be pathogenic for humans, and they are not known to produce toxic compounds or biogenic amines.

The most characteristic trait of AAB is the periplasmic oxidation of alcohols, aldehydes, sugars, and sugar alcohols in the presence of oxygen by dehydrogenases located on the outer surface of their cytoplasmic membrane.[57,63] For example, ethanol can be oxidized into acetaldehyde, which can be further oxidized into acetic acid. Glucose can be oxidized into gluconic acid, glucuronic acid, 2-ketogluconic acid, 5-ketogluconic acid, 2,5-diketogluconic acid, and glucuronic acid. In general, *Acetobacter* and *Gluconacetobacter* species prefer the oxidation of ethanol over glucose, whereas *Gluconobacter* species prefer the oxidation of glucose, glycerol, gluconic acid, and sorbitol over ethanol.[57,61] *Gluconobacter* species are indeed typically found in sugar-rich environments (such as flowers, fruits, honey bees), whereas *Acetobacter* and *Gluconacetobacter* species are usually associated with alcohol-rich environments. Upon depletion of ethanol, glycerol can be oxidized into **dih**ydroxy**a**cetone (DHA). This compound is known to react with the amino groups of amino acids and proteins to form a brown-colored complex and is used in skin tanning products.[64] DHA can also enter the Embden-Meyerhof-Parnas (EMP) pathway, and from there on the gluconeogenesis pathway, resulting in the production of cellulose. AAB do not have a complete functional EMP pathway (characterized by aldolase as the key enzyme) because they lack the enzyme phosphofructokinase, and metabolize glucose usually via the **p**entose **p**hosphate **p**athway (PPP; characterized by phosphoketolase as the key enzyme). *Acetobacter*, *Gluconacetobacter*, and *Komagataeibacter* species possess a complete functional TCA cycle, which allows them to completely oxidize (overoxidize) organic acids (such as acetic acid or lactic acid) into carbon dioxide.[61] This is in contrast with *Gluconobacter* species, which lack the enzyme α-ketoglutarate dehydrogenase and succinate dehydrogenase, and therefore cannot oxidize organic acids into carbon dioxide.

Many *Gluconacetobacter* and *Komagataeibacter* species produce water-insoluble cellulose polysaccharides [*β*-(1->4) glucans] from glucose, fructose, sucrose, and other substrates such as ethanol and glycerol.[65] This is clearly visible during a kombucha fermentation as a thick pellicle that develops on the surface of the fermentation liquor. Many factors impact the production of cellulose by AAB, such as type of substrate, substrate concentration, and pH.[66–68] Certain species of *Gluconobacter*, *Gluconacetobacter* and *Komagataeibacter* also produce water-soluble levans [*β*-(2->6) fructan] from sucrose, water-soluble dextrans [α-(1->6) glucans with α-(1->4) branches] from maltose or maltooligosaccharides, or water-soluble acetan polysaccharides (consisting of glucose, mannose, glucuronic acid, and rhamnose).[69–71]

Lactic acid bacteria

The presence of LAB in kombucha is inconsistent. Usually, they are not present [34,37] or present in low abundance,[14,24,31] but in certain industrial kombucha fermentations they have been found in higher abundance.[7] The

LAB recovered in the latter case were mainly *Oenococcus oeni* and *Lactobacillus nagelii*, both known to be acid-tolerant.[72]

LAB are gram-positive bacteria belonging to the phylum of the Firmicutes and are characterized by certain morphological, metabolic, and physiological characteristics.[73] They are not motile, are rod-shaped (*Lactobacillus*) or spherical (*Leuconostoc*, *Lactococcus*, *Oenococcus*), and do not sporulate. They are facultatively anaerobic, but do not use oxygen for their metabolism. Their optimal growth is ∼25-40 °C and pH 4.0-6.0. They are **g**enerally **r**egarded **as** **s**afe (GRAS), but some strains are known to produce biogenic amines, while on the other hand, many LAB are regarded as probiotic microorganisms.[74]

LAB are associated with the production of lactic acid, but they can also produce other metabolites such as ethanol, acetic acid, carbon dioxide, diacetyl, and mannitol.[73] LAB can be classified into obligately homofermenters (*Enterococcus*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Streptococcus*), obligately heterofermenters (*Leuconostoc*, *Lactobacillus*, *Oenococcus*, *Weisella*), and facultative heterofermenters (*Lactobacillus*). Homofermenters metabolize glucose via the EMP pathway with lactic acid as the main end product. Heterofermenters metabolize glucose via the pentose phosphate pathway with lactic acid, carbon dioxide, and ethanol as the main end products. When alternative electron acceptors (such as fructose) are present, acetic acid is produced instead of ethanol, whereby fructose is reduced into mannitol.[75] Facultative heterofermenters metabolize glucose via the homofermentative pathway, and metabolize pentoses and gluconic acid via the heterofermentative pathway. When cells have excess pyruvate available, for example due to the presence of external electron acceptors such as citrate in milk, pyruvate can be converted into α-acetolactate, which will spontaneously degrade into diacetyl.

LAB are well known for their ability to produce **exo**poly**s**accharides (EPS). A distinction is made between homopolysaccharides, which consist of only one monosaccharide and which can be produced in large quantities (g/L); and heteropolysaccharides, which consist of a repeating oligosaccharide (often containing glucose, galactose, and rhamnose) and which are usually produced in smaller quantities (mg/L).[76,77] Homopolysaccharides are synthesized by an extracellular transglycosylase (such as glucan- or fructansucrases) from a glycosidic donor (such as sucrose, lactose, or maltose) and a suitable acceptor molecule (such as maltose or growing polysaccharides). LAB EPS can be desirable in yogurt or sourdough bread for their stabilizing effects, but are usually undesirable in beverages, for example the production of EPS in beer by *Pediococcus damnosus*.

Others

The acidic environment of kombucha prohibits the growth of other microorganisms apart from those discussed above.[6,11] Nevertheless, members of the genera *Bifidobacterium*, *Thermus*, *Herbaspirillum*, *Halomonas* and others were found in kombucha via culture-independent techniques,[31,32] but it is unlikely that they play a key role during kombucha fermentation.

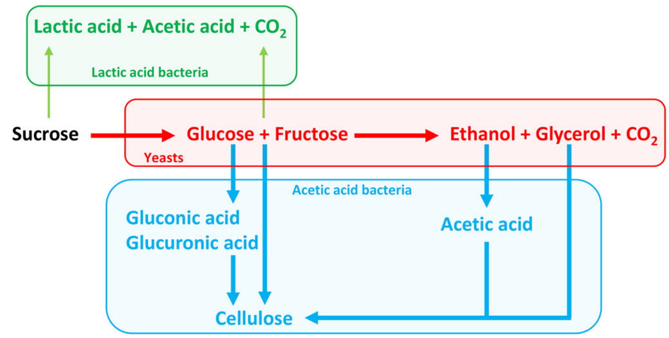

Figure 1. Main metabolic activities of yeasts, acetic acid bacteria, and lactic acid bacteria during a kombucha fermentation.

The kombucha fermentation process

Yeasts and AAB are both present during kombucha fermentation at around $10^6 - 10^8$ CFU/mL.[29,30,34,37] Extensive knowledge of the microbial diversity during kombucha fermentations has already been established, but the exact dynamics of the microbial ecosystem throughout the fermentation process has not yet been examined in sufficient detail and needs further investigation.

Within the kombucha microbial ecosystem, several symbiotic relationships can be found.[26] The substrate for a kombucha fermentation is usually sucrose, which is hydrolyzed into glucose and fructose by an invertase enzyme in the periplasm of yeast cells, whereby the concentrations of glucose and fructose increase (Figure 1).[34] This dynamic has also been described in detail for water kefir.[78] *Gluconobacter* species can also use sucrose, but *Acetobacter* and *Gluconacetobacter* species are dependent on yeasts for the hydrolysis of sucrose into glucose and fructose.[29,61] AAB (in particular *Gluconobacter* species) convert glucose into gluconic acid and glucuronic acid,[36] and yeasts appear to stimulate the production of glucuronic acid by AAB.[5]

Glucose and fructose are fermented by yeasts into ethanol, carbon dioxide, and glycerol (Figure 1).[29] The ethanol produced by yeasts is used by the AAB for the production of acetic acid.[29,34] In return, the production of acetic acid by AAB stimulates the production of ethanol by yeasts.[29] Furthermore, the presence of low concentrations of ethanol (around 1% v/v) stimulates the production of cellulose by the AAB.[66] The carbon dioxide will (at least partly) escape into the atmosphere, whereas glycerol can be oxidized by *Gluconobacter* species into DHA. Additionally, autolysis of yeast cells may stimulate the growth of AAB through the release of nutrients.[29] The production of cellulose by *Komagataeibacter xylinus* is thought to be proportional to its growth rate, whereas the final yield depends on the specific strain, the carbon source, and other factors.[61] The cellulose production itself appears to be stimulated by the presence of caffeine and other xanthines present in the tea.[61]

The main residual substrates in kombucha are fructose, glucose, and sucrose; and the main metabolites are ethanol, glycerol, acetic acid, gluconic acid, glucuronic acid, D-saccharic acid 1,4-lactone, and sometimes lactic acid.[11,29] However, quinic acid, oxalic acid, malic acid, and citric acid have also been reported.[37] The concentration of ethanol is reported to reach a maximum after 6-10 days of fermentation, after which it declines due to its conversion into acetic acid and/or cellulose,[38] but this is not always the case.[11,29,37] Final concentrations have been reported to be around 10 g/L ethanol, 3 g/L acetic acid, and 1 g/L glycerol.[29] However, conclusive data about the dynamics of the substrate consumption and metabolite production, as well as the final metabolite concentrations are not yet established, so more detailed investigations are still necessary.

During kombucha fermentations, not only symbiotic relationships develop, but also many selective pressures are at work that steer the microbial species diversity in kombucha, and consequently also impact the substrate consumption and metabolite production. For example, the production of ethanol by yeasts inhibits the growth of certain microorganisms.[79] One of the most important selective pressures during a kombucha fermentation is the acidic stress due to the production of organic acids by yeasts (acetic acid), AAB (acetic acid, gluconic acid, glucuronic acid), and sometimes LAB (lactic acid and acetic acid). The pH of kombucha tea starts at 5.0-7.0, and drops to 2.0-4.0 after 7 days of incubation, and can decrease to below 2.0 after extended incubation.[14,37,80] Kombucha fermented until high concentrations of acetic acid are present selects for *Komagataeibacter* species, as these microorganisms are known for their high tolerance of acetic acid.[81] The pH of industrial kombucha fermentation processes is probably more tightly controlled than household kombucha fermentations, as kombucha will become unpalatable below a certain pH. This also explains the presence of LAB in some industrial kombucha fermentation processes and their absence in household kombucha fermentation processes, as LAB do not grow below pH 3.5.[7]

Another important selective pressure that steers the microbial community is the limited availability of nutrients such as vitamins and nitrogenous compounds. The only sources of (micro)nutrients are the tea leaves used in the recipe. The low nutrient concentrations explain the prevalence of *Dekkera*/*Brettanomyces* species in kombucha, as these yeasts were found to be more competitive in a nutrient-poor environment.[82] *Dekkera bruxellensis* from kombucha did not produce glycerol and produced lower amounts of ethanol, but higher amounts of acetic acid in comparison with *Saccharomyces cerevisiae* from kombucha.[29] The nutrient limitation also explains the presence of nitrogen-fixing AAB in kombucha.[83,84]

The availability of oxygen will determine the growth of AAB, as these microorganisms are obligate aerobes. Initially, the tea will be completely oxygenated followed by a decreasing supply of oxygen, as oxygen is consumed by the AAB and its influx hampered by the growing cellulose pellicle on top of the liquid. It is therefore likely that the bottom part of the fermentation vessel will become anaerobic, limiting the conversion of ethanol and glucose into acetic acid and gluconic acid, respectively. Most yeasts, such as *Saccharomyces* and *Dekkera* species, will continue a fermentative metabolism as long as substrates are available, even under aerobic conditions.[43,45]

When glucose was used as a substrate, it seemed to be preferentially converted into gluconic acid and not into ethanol, whereas fructose seemed to be preferentially converted into ethanol.[38] Lactose did not seem to yield a satisfactory fermentation, as the pH remained > 5 after 17 days of incubation.[38] The optimal concentration of sucrose was around 50 g/L.[38] The best substrate for producing kombucha was found to be sucrose and black tea.[11] Other substrates such as glucose, fructose, and lactose did not result in a satisfactory fermentation, as well as other nutrient sources, such as green tea, peppermint tea, and lime blossom tea.

It is clear that the process parameters during a kombucha fermentation will exert a significant impact on the final microbial composition and therefore on the metabolite composition of kombucha. For example, the dimensions of the fermentation vessel and the ratio of the surface area to the volume could influence the level of oxygenation during a fermentation. This is less of a concern when the liquor is vigorously stirred or aerated during the fermentation process, as has been successfully applied by some producers. Low oxygenation and thus low acid production by AAB can result in a pH above 4, which might stimulate the growth of LAB and thus the production of lactic acid. Fermentation time will also have a substantial impact on the acidic stress to the microbial ecosystem and therefore the microbial and chemical composition of the final product. Higher fermentation temperatures appear to benefit LAB, which is consistent with their higher optimal growth temperatures in comparison to yeasts and AAB.[24]

Beverage composition

Substrates

Sucrose is converted into glucose and fructose during a kombucha fermentation. Neffe-Skocinska and colleagues[37] reported that fructose was consumed faster in kombucha than glucose, leaving glucose as the main residual carbohydrate in kombucha. However, these results are in contradiction with other carbohydrate research, where yeasts were reported to consume glucose almost twice as fast as fructose.[85] Fructose (relative sweetness 115-180) is sweeter than sucrose (relative sweetness 100), which is sweeter than glucose (relative sweetness 50-70).[86] However, the added sweetness from fructose (and to a lesser extent sucrose) decreases at higher concentrations, whereas the added sweetness from glucose and maltose increases at higher concentrations.[86] A certain amount of some sugars is probably necessary in kombucha to balance the sour taste.

Microbial metabolites

The kombucha beverage is characterized by the presence of organic acids. However, high concentrations of organic acids can render kombucha unpalatable and even dangerous to drink.[11] A temperature of 25 °C and 10 days of fermentation was found to be optimal to produce a kombucha with a good sensory quality.[37] The flavor profile of an organic acid depends on the pH level, whereby an acid is generally perceived as more sour at a lower pH, whereby the order of sourness between different acids remains constant at different pH levels.[87] Furthermore, the astringency of many organic acids increases at lower pH, so their astringency is a direct result of their acidic properties.[88]

Acetic acid is usually one of the prominent acids in kombucha. However, this acid has a rather harsh and pungent acidic taste and aroma,[87] which might not be desirable in large amounts in kombucha. Also gluconic acid is often found in kombucha and its sourness is described as mild, soft, and refreshing.[89] Gluconic acid can be further oxidized by AAB into 2-ketogluconic acid and 2,5-diketogluconic acid. Glucose can also be oxidized by kombucha AAB into glucuronic acid, which is thought to possess powerful detoxifying properties.[19] Furthermore, D-saccharic acid-1,4-lactone, a compound which is also thought to possess health-promoting properties, is produced during kombucha fermentation.[14,90–92] This compound is a competitive inhibitor of β-glucuronidase, an enzyme that promotes cancer formation by hydrolysis of conjugated glucuronides into cancerogenic aglyconic compounds in the bowel.[91] Lactic acid is not always found in kombucha, as LAB are not always present in kombucha. This acid possesses a mild sourness,[87] so the presence of LAB in kombucha may seem more desirable than the presence of AAB. However, LAB may pose some other challenges as they continue to produce acid after anaerobic packaging, whereas the metabolic activity of acetic acid bacteria stops after packaging under anaerobic conditions.

Ethanol and glycerol have a small, though inconsistent, positive impact on the body and viscosity of wine.[93] However, the ethanol concentration should remain below 0.5% (v/v) to sell kombucha as a soft drink. Glycerol has a relative sweetness of 80 and can increase the sweetness and smoothness of a beverage.[94,95]

Some AAB are able to produce a brown pigment, which may affect the color of the final kombucha beverage.[5,61] Furthermore, certain polymers that can be produced by acetic acid bacteria, such as cellulose, acetan, levan or other oligosaccharides may contribute to the sensory perception of kombucha, as is also the case for wine.[96] In addition, some of these polymers may provide benefits as prebiotic fibers.[97] On the other hand, their presence might be undesirable because they may hamper filtration.

Tea components

The tea used for brewing kombucha impacts the fermentation process and the sensory evaluation of the resulting kombucha beverage.[98,99] The most characteristic tea compounds are the catechins, and the concentrations of (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatecingallate (ECG), and (-)-epigallocatechingallate (EGCG) decrease over the course of a kombucha fermentation process,[34] as was also the case for the caffeine content.[14] Thearubigins are the brown pigments in black tea and theaflavins are the yellow pigments in black tea.[100] During kombucha fermentation, the concentrations of thearubigins decrease whereas those of theaflavins increase, probably because thearubigins are converted into theaflavins.[14,101] This may explain why kombucha becomes lighter in color (from dark brown tea to light brown kombucha tea) during the fermentation process. Overall, the total phenol content increases over the course of a kombucha fermentation process, as well as the antioxidant activity.[14,36,101,102]

Several tea compounds may also have an inhibitory effect towards certain yeasts, AAB, other microorganisms, and/or viruses and protozoa.[25,30] In contrast, caffeine and other xanthines may stimulate the synthesis of cellulose by AAB.[25]

Microorganisms

Kombucha attracts particular health-conscious consumers who tend to prefer an unfiltered and unpasteurized product, as they believe that certain kombucha microorganisms possess probiotic properties. However, the probiotic potential of kombucha seems to be rather limited, as the consumption of living yeast or AAB cells is not commonly associated with health benefits.[103] On the other hand, the presence of LAB may confer a health benefit to kombucha (depending on the specific strain), which would make their presence desirable. However, LAB do not appear to be an inherent member of the kombucha microbial community, and when LAB are present, their numbers seem to decline quickly during storage.[104]

The production of kombucha as an unfiltered and unpasteurized product poses many technological challenges, as the combination of living yeasts and bacteria with residual substrates creates an unstable product, even under refrigerated conditions. This can result in increasing alcohol concentrations and exploding bottles/cans as the alcoholic fermentation by yeasts continues. However, the presence of residual substrates may be necessary to obtain a well-balanced product. As a result, it is not uncommon for larger commercial kombucha brewers to employ sterile filtration or pasteurization technology prior to distributing their product into the market.

Future perspectives for kombucha

Currently, kombucha is still mainly produced via natural and therefore uncontrolled fermentation processes, often on a small scale. As the demand of the consumer for this beverage is increasing, the industry needs more reliable and detailed information about the kombucha fermentation process than what is currently available. To develop the next generation of kombucha fermentation processes, additional systematic scientific research is necessary. Especially consumer preferences for kombucha should be investigated in more detail to uncover their expectations. Furthermore, the creation of "designer kombuchas" may allow for a more predictable and economically viable fermentation process. The increasing interest in kombucha might stimulate more detailed scientific research on its health-promoting properties, which may in turn generate even more consumer interest in kombucha, depending on the outcomes.

Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORCID

David Laureys http://orcid.org/0000-0003-1175-5567
Scott J. Britton http://orcid.org/0000-0002-0874-3699
Jessika De Clippeleer http://orcid.org/0000-0002-8033-9308

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