Received: 7 January 2020 | Revised: 17 April 2020 | Accepted: 21 April 2020

DOI: 10.1111/1541-4337.12574

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

# Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha

**Thierry Tran**[1] | **Cosette Grandvalet**[1] | **François Verdier**[2] | **Antoine Martin**[2] | **Hervé Alexandre**[1] | **Raphaëlle Tourdot-Maréchal**[1]

[1]UMR Procédés Alimentaires et Microbiologiques, Université de Bourgogne Franche-Comté/AgroSup Dijon, Équipe Vin Alimentation Micro-organismes Stress (VAlMiS) Institut Universitaire de la Vigne et du Vin Jules Guyot, 2 rue Claude Ladrey, Dijon, 21000, France

[2]Biomère, 14 rue Audubon, Paris, 75012, France

**Correspondence**
Thierry Tran, UMR Procédés Alimentaires et Microbiologiques, Université de Bourgogne Franche-Comté/AgroSup, Dijon, Équipe Vin Alimentation Micro-organismes Stress (VAlMiS) Institut Universitaire de la Vigne et du Vin Jules Guyot, rue Claude Ladrey, BP 27877, 21000, Dijon, France.
Email: thierrytran01@gmail.com

**Funding information**
European Regional Development Fund; Biomère

**Abstract**

Kombucha is a beverage made from sugared tea transformed by yeasts and acetic acid bacteria. Being originally homemade, it has become an industrially produced soft drink whose quality standards are poorly defined and whose production process is still not fully controlled. Based on current knowledge in beverages, links between kombucha's chemical composition and sensorial compounds are drawn. Macromolecules create turbidity, whereas uncharacterized tea pigments derivatives participate in the color. Residual sugars bring sweetness and organic acids produced by acetic acid bacteria form its characteristic sour taste. Acetic acid is also part of its aroma profile, although little data are available on the smell of kombucha. Carbon dioxide, potentially polyphenols, and residual ethanol are involved in the mouthfeel. In this review, after defining the key compounds that shape the characteristic sensory properties of kombucha, the impact of different production parameters is discussed. Water composition is determinant in the extraction of tea compounds along with the tea type and infusion duration and temperature. The type and amount of sweeteners play a role in the sweetness and influences the production kinetics. Similarly, the amount of inoculum and its microbial composition have an effect on the production, but the role of the vessels' geometry and temperature are also essential parameters that can be used to adjust the acidification phase's duration. Despite the amount of research carried out, further investigations of kombucha's sensory characteristics are needed. Such research could lead to a better definition of kombucha's quality and to an improved control over its production process.

KEYWORDS
fermentation, Kombucha, process, quality, sensory

## 1 | INTRODUCTION

Kombucha, also named "kombucha tea," is a fermented beverage resulting from the activity of a microbial consortium including yeasts, acetic acid bacteria, and often (but not always) lactic acid bacteria in sugared tea liquor as liquid medium (Dufresne & Farnworth, 2000; Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014; Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018). It is believed to originate from Asia but all the elements appearing in the scientific literature, especially about the Chinese origins of kombucha, are supported by unverified sources (Dufresne & Farnworth, 2000; Troitino, 2017). Nevertheless, these stories continue to feed the mythology of kombucha and could be used to support the marketing communication of industrialized products. Indeed, the main marketing arguments of kombucha are its putative benefits for human health. Numerous *in vivo* and *in vitro* studies were carried out to establish the existence of antioxidant (Bhattacharya, Gachhui, & Sil, 2013; Gamboa-Gómez et al., 2016; Jayabalan, Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008), antimicrobial (Battikh, Bakhrouf, & Ammar, 2012; Sreeramulu, Zhu, & Knol, 2000; Steinkraus, Shapiro, Hotchkiss, & Mortlock, 1996), and hepato-protective effects (Murugesan, 2009; Wang et al., 2014). Nevertheless, the existence of beneficial effects of kombucha for human health remains controversial because of the insufficient amount of decisive scientific data (Ernst, 2003; Jayabalan et al., 2014; Martínez Leal, Valenzuela Suárez, Jayabalan, Huerta Oros, & Escalante-Aburto, 2018) and because of multiple cases of diseases or unexplained death following the overconsumption of kombucha (Holbourn & Hurdman, 2017; Phan et al., 1998; Sung, Kole, Jones, Christensen, & Gladstein, 2009). The active compounds of kombucha with potential benefits for human health originate from tea polyphenols, in particular epigallocatechin gallate (Jayabalan, Marimuthu, & Swaminathan, 2007; Khan & Mukhtar, 2007), hydrolytic enzymes, vitamins ($B_1$, $B_2$, $B_6$, $B_{12}$, and C) (Bauer-Petrovska & Petrushevska-Tozi, 2000; Kumar & Joshi, 2016), and organic acids such as gluconic acid, glucuronic acid (Nguyen, Dong, Le, & Nguyen, 2014; Nguyen, Nguyen, Nguyen, & Le, 2015) or D-saccharic-1,4-lactone acid (Wang, Gan, Tang, Wang, & Tan, 2010) produced by the microorganisms. To preserve these potential benefits and/or promote a clean label, some kombucha producers do not pasteurize nor filter their products.

During the last decades, kombucha transitioned from a homemade fermented beverage to a commercialized soft drink produced industrially. A striking result is that the market of kombucha is expected to exhibit a strong growth rate of 17.5% in the United States between 2019 and 2024 (Mordor Intelligence, 2019).

Despite a strong development of kombucha-producing companies, the producers of this beverage suffer from a lack of technical knowledge similar to what exists for the production of other beverages, such as wines or beers. This review aims at giving the kombucha brewers, R&D staff, and researchers an innovative approach of kombucha as a commercialized fermented beverage. It will revolve around the identification and control of the different dimensions, or components, of kombucha's quality through the lens of the current available scientific literature. The concept of quality developed on wine was studied by Charters and Pettigrew (2007) and distinguished between extrinsic and intrinsic qualities. This review will focus on the intrinsic quality of tea-based kombucha that is bound to its structural features, in other words its sensory properties. This then excludes all the aspects related to price, packaging, or marketing. Also, fermented products based on infusions of plants that are not tea (*Camellia sinensis*) or on other food matrices will not be discussed, nor will flavored kombuchas. Indeed, the use of aromatization ingredients such as fruits extracts, herbs, or spices implies the addition of a variety of compounds. Among them, nitrogen sources, sugars, or antimicrobial compounds can be the origin of significant modification in microbial dynamics and elaboration kinetics before and after bottling. The complexity of the effects that could be induced by the addition of such ingredients makes inclusion of this topic injudicious and thus it is not addressed in the present review. For the review on kombucha made with plants other than tea, the reader can be directed to the review of Emiljanowicz and Malinowska-Pańczyk (2019).

After defining kombucha and the way it is generally produced, due to the lack of published sensory data on kombucha beverage, the components of kombucha's flavor will be discussed by breaking down its chemical composition and discussing the known relationship of these parameters with sensory characteristic. Then, with all these elements in mind, different aspects of the production process will be presented in order to give the reader all the known levers that can be used to shape the product's flavor and quality.

## 2 | GENERAL KNOWLEDGE ABOUT KOMBUCHA AND ITS PROCESS

The traditional way of making kombucha consists in the brewing of black tea liquor to which sucrose is added. Most of the scientific literature reports the use of infusion (initial temperature between 70 and 95 °C) rather than decoction in order to perform tea extraction (Ali & Shivanna, 2017; Dufresne & Farnworth, 2000; Jayabalan et al., 2014; Villarreal-Soto et al., 2018). Thus, infusion will be used as the initial process in the context of the present review. After the liquid reaches room temperature, the infusion is inoculated with a kombucha culture in the form of a pellicle fragment or as whole (traditionally referred to as "tea fungus") and/or broth (Dufresne & Farnworth, 2000; Greenwalt, Steinkraus, & Ledford, 2000; Jayabalan et al., 2008). In the course of kombucha production a new cellulosic pellicle forms itself at the surface of the liquid phase (Chen & Liu, 2000). Despite the lack of consensual definition of a biofilm, the pellicle satisfies the definition of a biofilm with aggregated and sessile cells without a solid surface (Alhede et al., 2011). In the case of kombucha, the cellulosic pellicle is an air–liquid interface biofilm. Such structure has already been reported by two studies focusing on the wine matrix (David-Vaizant & Alexandre, 2018; Zara et al., 2005). Kombucha pellicle will therefore be referred to as "biofilm" in the context of the present review.

The different steps of kombucha elaboration are not standardized. According to previous studies, sucrose concentration and tea amount can range from 50 to 100 g/L and from 1.5 to 10 g/L, respectively, with steeping time between 5 and 15 min (Blanc, 1996; Chen & Liu, 2000; Chu & Chen, 2006; De Filippis, Troise, Vitaglione, & Ercolini, 2018; Goh et al., 2012a; Jayabalan et al., 2014; Kallel, Desseaux, Hamdi, Stocker, & Ajandouz, 2012; Lončar, Djurić, Malbaša, Kolarov, & Klašnja, 2006; Malbaša et al., 2006; Malbaša, Lončar, & Djurić, 2008; Neffe-Skocińska, Sionek, Ścibisz, & Kołożyn-Krajewska, 2017; Reiss, 1994; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995). Namely, the order and length of each step and the various amounts of tea, sugar, and inoculum can vary and be adapted depending on personal and empirical appreciations.

To trigger the transformation of sugared tea infusion in kombucha, a microbial culture must be added to the sweetened tea medium. Jayabalan et al. (2014) mention, while describing the inoculation process, that kombucha culture as tea fungus has to be placed in the sugared tea broth. Nevertheless, whether the process is home-made, produced at industrial scale, or in research labs, several methods are reported. For example, the inoculation step has been reported to be achieved by the addition of the broth (Blanc, 1996; Jayabalan et al., 2007; Loncar, Kanuric, Malbasa, Djuric, & Milanovic, 2014; Malbaša et al., 2008), the addition of only the biofilm or biofilm fragments (Jayabalan, Malini, Sathishkumar, Swaminathan, & Yun, 2010; Reiss, 1994; Sievers et al., 1995), or the addition of both (Chen & Liu, 2000; Goh et al., 2012a; Kallel et al., 2012).

There is no single "culture" or microbial consortium for developing kombucha but instead a multitude of matrix-dependent consortia whose origins are unknown. It appears that the only constant element that defines a kombucha culture is the simultaneous presence of yeasts and acetic acid bacteria, lactic acid bacteria not being always present.

The acetic acid bacteria community is mostly represented by the genera : *Acetobacter* (sp. *okinawensis* and *tropicalis*), *Gluconobacter* (sp. *oxydans*), *Gluconacetobacter* (sp. *europaeus* and *saccharivorans*), and *Komagataeibacter* (sp. *kombucha* and *xylinus*) (Chakravorty et al., 2016; De Filippis et al., 2018; Marsh, O'Sullivan, Hill, Ross, & Cotter, 2014; Reva et al., 2015).

The yeast community is more variable and includes genera such as *Zygosaccharomyces* (sp. *lentus*, *bisporus* and *bailii*), *Candida* (sp. *stellimalicola* and *tropicalis*), *Lachancea* (sp. *thermotolerans* and *fermentati*), *Kloeckera/Hanseniaspora* (*sp. valbyensis), Torulaspora* (sp. *delbrueckii)*, *Rhodotorulaspora* (sp. *mucilagenosa*), *Pichia* (sp. *mexicana* and *occidentalis*), *Brettanomyces/Dekkera* (sp. *bruxellensis* and *anomala*), *Saccharomyces* (*sp. cerevisiae*), *Schizosaccharomyces* (sp. *pombe*) and *Saccharomycoides* (Chakravorty et al., 2016; Coton et al., 2017; De Filippis et al., 2018; Markov, Cvetković, & Bukvić, 2005; Marsh et al., 2014; Reva et al., 2015; Teoh, Heard, & Cox, 2004).

Other microbial families have been identified and involve lactic acid bacteria with namely the genera *Lactobacillus*, *Bifidobacterium*, and *Oenococcus* (Chakravorty et al., 2016; Coton et al., 2017; Marsh et al., 2014).

The biological transformation driving the elaboration of kombucha is a combination of microbial metabolic interrelationships, which is not limited to a plain fermentation. Consequently, this microbial process will be referred to as "elaboration" in the context of the present review. Kombucha elaboration occurs generally following the process shown in Figure 1. At room temperature, the sucrose added in the tea liquor is hydrolyzed by the yeasts into fructose and glucose, which are subsequently converted into ethanol through alcoholic fermentation. This step is the basis of the symbiosis that occurs in kombucha because acetic acid bacteria are unable to metabolize sucrose. But yeasts provide them carbonated substrates that they can use. Since the system is in contact with air, the obligate aerobe acetic acid bacteria use the available glucose and ethanol to produce organic acids (Chen & Liu, 2000; Sievers et al., 1995), mainly acetic acid from the oxidation of ethanol and gluconic acid obtained from glucose as part of their oxidative metabolism (De Ley, 1961; Lynch, Zannini, Wilkinson, Daenen, & Arendt, 2019). Although glucose is used by acetic acid bacteria, it is thought that a part can remain available for yeasts as well as fructose as substrates for alcoholic fermentation (Sievers et al., 1995). The acidification of the medium leads to the decrease of pH, which prevents the development of pathogenic microorganisms under the value of 3 (Leistner, 2000). It is worth noting that the initial pH after inoculation is dependent on the total acidity of the inoculum. In parallel, the formation of a gelatinous mass is observed. This biofilm composed of cellulose is produced by some acetic acid bacteria, more specifically *Komagataeibacter xylinus* (Zhang, Wang, Qi, Ren, & Qiang, 2018) (formerly named *Acetobacter xylinum* and *Gluconacetobacter xylinus* [Yamada et al., 2012]). In a context of industrial production, the biofilm might be removed before bottling and smaller pieces can be eliminated by filtration. After bottling, further cellulose synthesis should be stopped because of oxygen deprivation after consumption of residual oxygen by microorganisms, namely acetic acid bacteria or yeast with respiratory metabolism. Similarly, the acetic conversion can be stopped at any time according to the desired taste of the beverage by putting the system in anaerobic conditions, which inhibits acetic acid bacteria and favors alcoholic fermentation of yeasts, turning the residual sugars into ethanol and carbon dioxide. A sour and sparkling beverage is obtained. To our knowledge, little investigation has been carried out on this secondary fermentation. Since different elaboration times are used, chemical compositions reported in the literature are very diverse (Table 1 [Villarreal-Soto et al., 2018]). In order to provide a good acceptability of the product, the brewer will

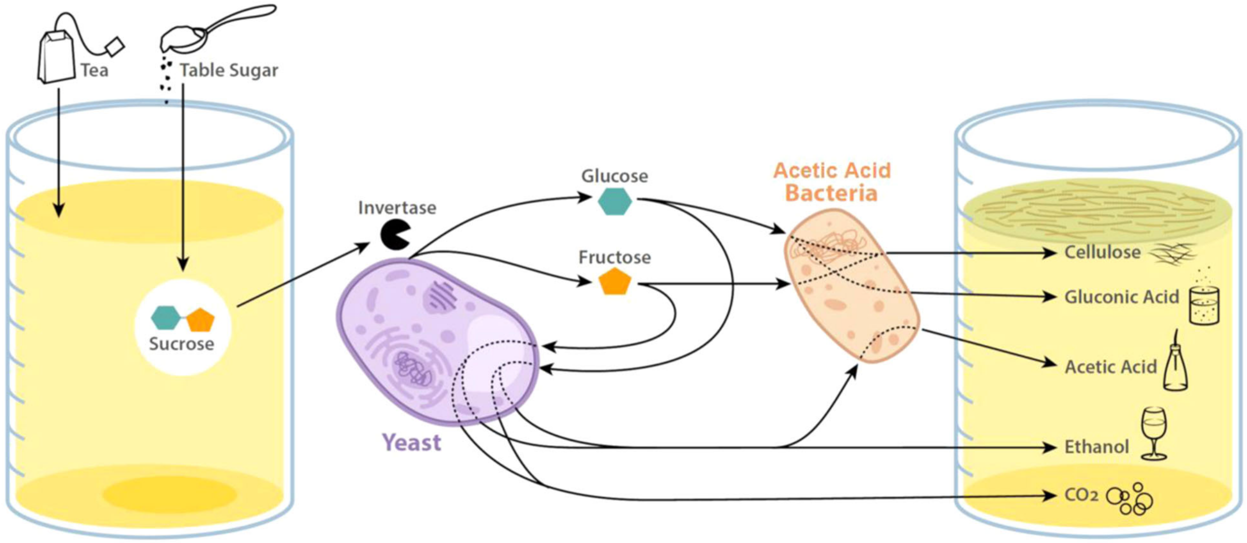

**FIGURE 1** Kombucha metabolism and microbial interactions. (a) Kombucha is brewed by adding tea and table sugar to a small amount of kombucha starter that contains yeast and acetic acid bacteria. These microbes begin to break down the sugar, leading to a metabolic cascade that ends with a bubbly, acidic, and slightly alcoholic beverage. (b) During the process of elaboration, cooperative and competitive interactions occur among microbes. The production of the public good invertase by yeast, the removal of waste products through metabolization of alcohol, and the generation of the cellulose pellicle by bacteria are potentially cooperative functions. Antimicrobial metabolites, low pH, and the generation of a physical barrier inhibit the growth of competitors (adapted from May et al., 2019)

**TABLE 1** General chemical composition of Kombucha (Villarreal-Soto et al., 2018)

| | Compound | Average composition | Initial sucrose | Fermentation time (days) | References |
|---|---|---|---|---|---|
| Organic acids | Acetic acid | 5.6 g/L | 70 g/L | 15 | Blanc(1996) |
| | Acetic acid | 8.36 g/L | 100 g/L | 18 | Jayabalan et al. (2007) |
| | Acetic acid | 11 g/L | 100 g/L | 30 | Chen and Liu (2000) |
| | Gluconic acid | 39 g/L | 100 g/L | 60 | Chen and Liu (2000) |
| | Glucuronic acid | 0.0160 g/L | 70 g/L | 21 | Lončar et al. (2006) |
| | Lactic acid | 0.18 g/L | 100 g/L | 18 | Jayabalan et al. (2007) |
| Vitamins | Vitamin $B_1$ | 0.74 mg/L | 70 g/L | 15 | Bauer-Petrovska and Petrushevska-Tozi (2000) |
| | Vitamin $B_2$ | 8 mg/100 mL | 70 g/L | 10 | Malbaša et al. (2011) |
| | Vitamin $B_6$ | 0.52 mg/L | 70 g/L | 15 | Bauer-Petrovska and Petrushevska-Tozi (2000) |
| | Vitamin $B_{12}$ | 0.84 mg/L | 70 g/L | 15 | Bauer-Petrovska and Petrushevska-Tozi (2000) |
| | Vitamin C | 25 mg/L | 70 g/L | 10 | Malbaša et al. (2011) |
| General composites | Ethanol | 5.5 g/L | 100 g/L | 20 | Chen and Liu (2000) |
| | Proteins | 3 mg/mL | 100 g/L | 12 | Jayabalan et al. (2007) |
| | Tea polyphenols | 7.8 mM (gallic acid equivalent) | 100 g/L | 15 | Chu and Chen (2006) |
| Minerals | Cu, Fe, Mn, Ni, Zn | 0.1 to 0.4 $\mu$g/mL | 70 g/L | 15 | Bauer-Petrovska and Petrushevska-Tozi (2000) |
| Anions | $F^-$, $Cl^-$, $Br^-$, $I^-$, $NO_3^-$, $HPO_4^-$, $SO_4^-$ | 0.04 to 3.20 mg/g | 100 g/L | 7 | Kumar and Joshi (2016) |

have to make sure that the acidity is not too high and the pH not too low in the case of long-lasting elaboration, else the taste will not remain pleasant. The pH value of 3 appears to be a minimum threshold for kombucha acceptability; a lower pH would be too acidic (Lončar et al., 2006).

The microbiological studies of kombucha elaboration show an increase in yeast and bacteria population during the two first days following the inoculation of the sugared tea liquor (Coton et al., 2017; De Filippis et al., 2018; Teoh et al., 2004). Next, different variations in populations were reported according to the consortium used (Chen & Liu, 2000), the type of tea (Coton et al., 2017), or the temperature (De Filippis et al., 2018). The impact of these parameters on the process and the final product will be developed in Section 4.

The microbial dynamics occurring in a given kombucha consortium during the elaboration highlighted the domination of one to three genera for the yeasts (Chakravorty et al., 2016; Reva et al., 2015; Teoh et al., 2004) and for bacteria (Chakravorty et al., 2016; Coton et al., 2017; Reva et al., 2015) over the important diversity of the other detected genera (with an abundance inferior to 1% per genus). One predominant genus is often found per kingdom, for example: *Candida stellimalicola*, (Chakravorty et al., 2016) or *Dekkera anomala* (Reva et al., 2015) for the yeasts and *K. xylinus* (Reva et al., 2015) for the bacteria. Some studies have reported important variations of the proportion of the dominant species over time, suggesting the occurrence of interaction between yeast species (Chakravorty et al., 2016; Teoh et al., 2004) and between bacteria species (Coton et al., 2017).

Currently, the control of kombucha production is mostly empiric despite the increasing amount of knowledge and understanding provided by the scientific community. Yet, professional producers of kombucha are confronted with several difficulties for the control of its elaboration. First, there exists a wide variability of elaboration kinetics due to the complex and hard-to-control microbial consortium, as opposed to a single culture fermentation (Villarreal-Soto et al., 2018). Moreover, the production of batches by successive inoculations (also called propagation) could lead to a modification or evolution of the consortium in terms of composition, microbial dynamics, or both. Finally, in the current context, refrigeration is not always sufficient to completely prevent microbial activity after commercialization of unstabilized bottled kombucha (using thermal or filtration processes). Because of the possibility of yeast refermentation, the production of carbon dioxide resulting in bottle explosions is a real risk for kombucha producers. Moreover, the possible increase in alcohol content (Talebi, Frink, Patil, & Armstrong, 2017) could have consequences on regulatory levels. In fact, kombucha is classified as nonalcoholic beverage only as long as its alcohol content does not exceed a threshold value. In United States, it is set at 0.5% (Code of Federal Regulations, Title 27: Alcohol, Tobacco and Firearms [Alcoholic content, 1993]), whereas in the European Union, this limit is set at 1.2% (Regulation [Eu] No 1169/2011 Of The European Parliament And Of The Council [Official Journal of the European Union, 2011]).

## 3 | DISSECTION OF THE CHEMICAL COMPOSITION OF KOMBUCHA IN RELATIONSHIP TO ITS POTENTIAL SENSORIAL IMPACT

The perception of all food and beverages is conveyed by the consumer's five senses: sight, hearing, touch, smell, and taste. When drinking kombucha, the consumer experiences a mix of visual (aspect of the product), olfactive (aroma profile before and after ingestion), taste (sweetness, sourness), and touch sensations (for example, *via* chemosensation of the tingling of carbon dioxide bubbles on the tongue) (Redondo, Gomez-Martinez, & Marcos, 2014).

There are very few data available of descriptive sensory analysis on tea kombucha beverages. The study of Neffe-Skocińska et al. (2017) includes a sensory analysis of kombucha beverages elaborated during 10 days, made of a mix of black and green tea (2 and 4 g/L, respectively) and 100 g/L sucrose at 20, 25, and 30 °C. The sensory analysis was performed by a panel of 16 untrained people and no statistical analysis was performed on the results obtained (Figure 2 [Neffe-Skocińska et al., 2017]). Therefore, the interpretation of these results should be handled with care. Nevertheless, the descriptors used are representative of the sensory characteristics of kombucha and help to define its main features. The descriptors with highest scores are: "color intensity" and "clarity" for the visual attributes; "tea" and "citric" for the olfactory attributes; and "tea," "citric," and "acid" for the flavor attributes. The descriptor "sweetness" was not assessed despite residual sugars ranging from 27 to 70 g/L. The descriptors "yeast flavor," "acetic acid flavor," "bitter flavor," "stinging flavor" have low scores. The low "stinging flavor" score could be explained by a lack of secondary fermentation in closed vessels. Recent studies incorporated sensory analysis but did not bring further detailed descriptive elements (Ivanišová et al., 2019; Shahbazi, Hashemi Gahruie, Golmakani, Eskandari, & Movahedi, 2018).

The use of a more precise and standardized set of descriptors could allow deeper investigations of the olfactive and gustative dimensions of kombucha. So, a more indirect approach is necessary to explore the sensory characteristics of kombucha by relating the chemical constituents of kombucha to the known sensory properties of those compounds.

### 3.1 | The sight: the aspect of kombucha

Kombucha beverages can be either clear filtered or turbid (as nonfiltered version). The turbidity of the latter is mainly due to the colloidal state of the aqueous beverage, defined as a suspension of particles. These particles are composed of

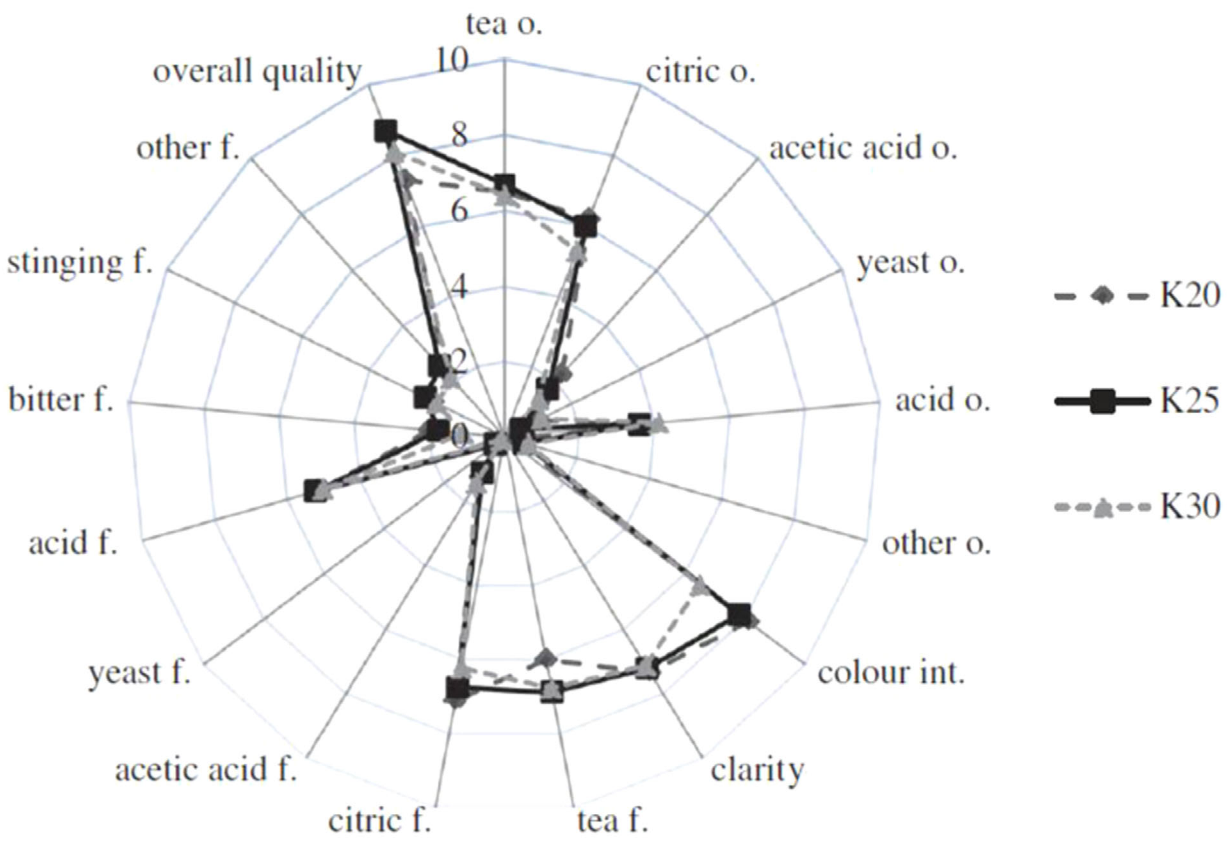

**FIGURE 2** Sensory profiles of the kombucha beverages after 10 days of elaboration process at 20, 25, and 30 °C (K20, K25, and K30, respectively). "o." stands for "olfactive," "int." stands for "intensity," and "f." for "flavor" (Neffe-Skocińska et al., 2017)

microorganisms and large molecules or aggregates ranging from 1 to 1000 nm. The presence of such bodies induces the scattering of the light. This phenomenon is known as the Tyndall Effect (Petrucci, Herring, Madura, & Bissonnette, 2011). Although little investigation was carried on the colloids of kombucha, it can be speculated that they can result from the aggregation of proteins (Jayabalan et al., 2007; Petrović, Suturović, Lončar, & Malbaša, 1999), polyphenols, and cellulose fibrils produced by acetic acid bacteria (Goh et al., 2012b; Lin et al., 2013; Zhang et al., 2018). Colloids play a huge part of soft drinks' intrinsic quality (Kappes & Schmidt, 2007), but to our knowledge there are no data available mentioning the mouthfeel of kombucha.

The color hue of kombucha is mainly due to the presence of the pigment polyphenols extracted from the tea. The characteristic color of black tea results from polyphenols oxidase-type enzymes, or the so-called "fermentation" of fresh tea leaves (Harris & Ellis, 1981). This process allows the oxidation and polymerization of native polyphenols composed mainly of catechins (epicatechin, epigallocatechin, and their gallic acid ester derivatives) into different classes of polymers (Balentine, Wiseman, & Bouwens, 1997; Harbowy, Balentine, Davies, & Cai, 1997). Two of those classes are pigments: one of them is theaflavin, a red-orange pigment dimer that gives black tea its characteristic color (Harbowy et al., 1997). Nonetheless, theaflavin is not the main contributor of black tea's color. The second family named thearubigins, results from a higher degree of polymerization and acts as the main pigment. The molecular structure of those polymeric molecules is still not fully elucidated (Haslam, 2003). Chemical structures of some of those compounds are displayed in Figure 3.

Although little attention was given to the color and polyphenols of kombucha, two studies reported a significant decrease in color intensity and increase in total phenolic content during kombucha elaboration (Chakravorty et al., 2016; Chu & Chen, 2006). The decrease of pH could be the cause of the change of color (United States Patent No· 4,552,776, 1985). It has also been hypothesized that the biological activity of the kombucha consortium may alter or even depolymerize the pigments extracted from the tea (Haslam, 2003) Thus, a large part of kombucha pigments may be derivatives from the tea polyphenols.

Another component of kombucha's visual identity as a carbonated beverage is the aspect and abundance of the bubbles that are dependent on the carbonation process that can be obtained naturally after fermentation in closed vessel or by injecting carbon dioxide artificially (forced carbonation)

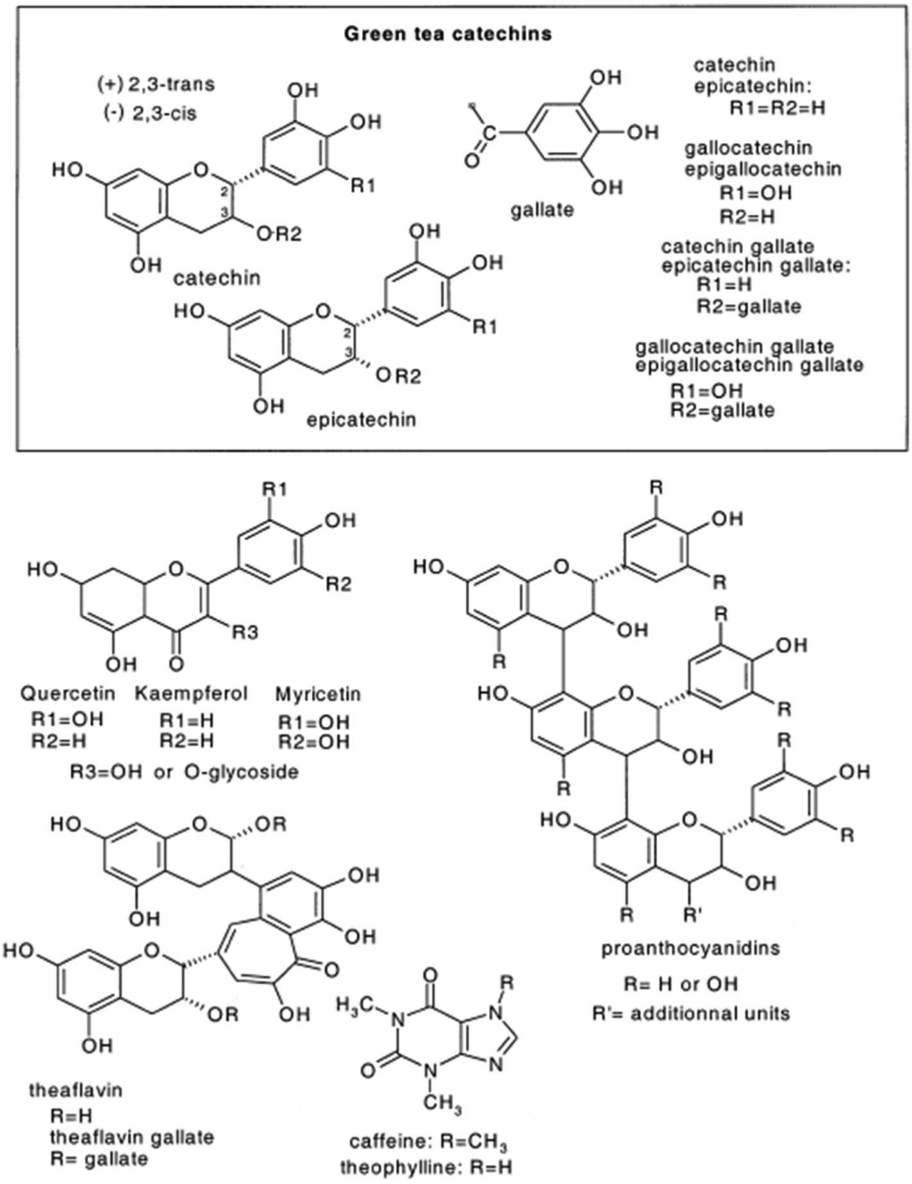

**FIGURE 3** Chemical structure of some tea constituents (adapted from Dufresne and Farnworth [2000])

(Barker, Jefferson, & Judd, 2002; Descoins, Mathlouthi, Le Moual, & Hennequin, 2006). Bubbles number is dependent on nucleation sites, which mainly consist in particles (belonging to the product itself or its vessel) (Lubetkin & Blackwell, 1988; Wilt, 1986). The formation kinetics and size of bubbles is dependent on the interfacial tension between the gas and liquid phase (Jones, Evans, & Galvin, 1999), which means that macromolecules such as polysaccharides and proteins play a role in the visual aspect of a carbonated drink (Barker et al., 2002).

## 3.2 | The smell: a vaporous idea of kombucha's aroma profile

Little information is available about the volatile compounds of kombucha, their origins, and their relationship to olfactory experience (as for most food and beverages) (Acree & van Ruth, 2003), which is why this section relies mainly on speculations. Kombucha's smell has been widely described as "cidery" (Dufresne & Farnworth, 2000; Greenwalt et al., 2000; Jayabalan et al., 2014). As many fermented beverages, the odorant compounds originate from both the raw material (the tea) and volatile metabolites produced by the microorganisms. Although black tea hosts numerous volatile molecules such as 3-hexenol (greenish), linalool (floral), geraniol (sweet, honey-like), 2-phenylethanol (honey-like), damascenone (rose-like), or 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) (caramel-like) (Ho, Zheng, & Li, 2015; Robinson & Owuor, 2013; Teranishi, Wick, & Hornstein, 1999), typical tea aroma never seems to be part of the characteristic aroma profile of kombucha. Instead, the elaboration-related aromas dominate: the vinegary odor associated with acetic acid produced by acetic acid bacteria and the cidery odor associated with the activity of yeasts (Rosend, Kuldjärv, Rosenvald, & Paalme, 2019; Wei, Wang, Zhang, Yuan, & Yue, 2019). In cider, higher alcohols are largely produced: amyl alcohols (banana, pear), butanol (balsamic), propanol (fermented, fruity), ethyl acetate (solvent, fruity like), and ethyl lactate (creamy, fruity). 2-phenylethanol, hexanol (green), octanol (citrus), and butanoic acid (cheesy) were also identified in a significant amount (Mangas, González, Rodríguez, & Blanco, 1996; Rosend et al., 2019; Valles, Bedrinana, Tascon, Simon, & Madrera, 2007; Williams & Rosser, 1981).

It is noteworthy that many yeast genera are common to both kombucha and cider, namely *Candida*, *Hanseniaspora*, *Pichia*, *Dekkera*, and *Saccharomyces* (Morrissey, Davenport, Querol, & Dobson, 2004; Valles et al., 2007; Wei et al., 2019). Although it can be suggested that the production of volatile metabolites follows similar pathways, there are currently too few elements to make any further statement on this topic. Thus, many questions remain open: what are the volatile compounds essential to kombucha aroma and what are their origins? If a comparison with wine is conducted, the deglycosylation of some aroma precursors by yeast $\beta$-glucosidase during fermentation might have very little impact on kombucha (Fia, Giovani, & Rosi, 2005; Hernandez, Espinosa, Fernandez-Gonzalez, & Briones, 2003). The enzymatic oxidation of polyphenols (see Section 3.1.) can lead to aroma release in black tea before kombucha elaboration (Ho et al., 2015; Zhou et al., 2017). On the contrary, the precursors are present in green tea in their glycosylated form, which means that the use of this raw material for kombucha elaboration could unlock aroma potential through the enzymatic activity of yeasts.

## 3.3 | The taste: a complex combination of sapid substances

### 3.3.1 | Sweetness

The traditional sweetener used for kombucha, which also acts as carbonated substrate for the microorganisms, is sucrose (Blanc, 1996; Dufresne & Farnworth, 2000; Jayabalan et al., 2014; Villarreal-Soto et al., 2018). The use of sucrose constitutes the basis of the symbiosis between yeast and bacteria, the former breaking down sucrose *via* the activity of invertase into glucose and fructose that are usable by the latter (May et al., 2019). It is possible to bypass this necessary step for sucrose hydrolysis by introducing sugars in the form of purified or mixed ingredients such as glucose syrup, agave syrup, or molasses (Malbaša et al., 2008; Reiss, 1994). These ingredients differ on two major aspects. The first is the difference in relative sweetness: glucose and fructose possess a sweetness intensity of 65 to 75% and 120% (w/w), respectively, compared to sucrose (although those values are modulated by their concentrations [Stone & Oliver, 1969]). The second is other compounds contained in those ingredients, such as those produced by the Maillard reactions in agave syrup or molasses (Willems & Low, 2012). These compounds can be volatile impacting the aroma profile or nonvolatile such as minerals or pigments that can impact the visual aspect of the beverage.

The sweetness of the final product is thus dependent on the residual amount of sugars, which is conditioned by the initial amount of sweetener added to the tea and the consumption of this substrate by microorganisms during the elaboration. More details about the influence of the substrate and its initial quantity on elaboration are developed in Section 4.3.

### 3.3.2 | Sourness

Organic acids of kombucha are mainly produced by acetic acid bacteria, although the contribution of yeasts and lactic acid bacteria should not be neglected. The major organic acids contributing to kombucha's taste are acetic acid, gluconic acid, and glucuronic acid, whereas the minor ones are lactic acid, malic acid, and succinic acid (Blanc, 1996; Chakravorty et al., 2016; De Filippis et al., 2018; Jayabalan et al., 2007; Malbaša et al., 2008; Neffe-Skocińska et al., 2017). The sensory properties of these metabolites are detailed in Table 2.

It should be noted that organic acids also generate, on a lower level, a bitter and astringent taste (Rubico & McDaniel, 1992; Siebert, 1999). Yet, those chemical species are not the main origin of the bitter perception of kombucha.

### 3.3.3 | Bitterness

Bitterness in kombucha, if not masked by the sweetness, can take origin from tea caffeine and polyphenols (Balentine et al., 1997; Harbowy et al., 1997). The average perception taste threshold for caffeine has been reported of 0.2 g/L (Paulus & Reisch, 1980) and Chakravorty et al. (2016) reported a caffeine content ranging above this value from 0.6 to 1 g/L in the course of black tea kombucha elaboration.

Polyphenols are bitter and astringent secondary metabolites produced by plants, including tea (*C. sinensis*) (Harbowy et al., 1997; Lesschaeve & Noble, 2005). Before any treatment, tea leaves are composed of 30 to 40% (w/w dry weight) of polyphenols. After infusion in hot water, the proportion in dry matter extracted is about the same (Balentine et al., 1997; Harbowy et al., 1997). The phenolic composition of a green tea infusion can be summed up (in weight percentage of solid extract) as a majority of catechins (30 to 42%): epicatechin, epigallocatechin, and their gallic acid esters (epicatechin gallate and epigallocatechin gallate). Minor compounds (2%) are represented by flavonols (kaempferol, quercetin, and myricetin) as aglycones and glycosides. Phenolic acids such as gallic acid and theogallin are also found (2% in

**TABLE 2** Chemical and sensorial properties of organic acids of kombucha (Da Conceicao Neta, Johanningsmeier, & McFeeters, 2007; Li & Liu, 2015; Ramachandran, Fontanille, Pandey, & Larroche, 2006)

| Acid | Molecular weight (g/mol) | pKa | Number of carboxylic functions | Taste perception threshold (mg/L water) | Sensory quality |
|---|---|---|---|---|---|
| Acetic | 60 | 4.75 | 1 | 52.6 | Tart and sour |
| Lactic | 90 | 3.86 | 1 | 80.1 | Acrid |
| Gluconic | 196 | 3.86 | 2 | Not determined | Mild, soft, refreshing taste |
| Malic | 134 | 3.40 and 5.11 | 2 | 7.3 | Smooth tartness |
| Succinic | 118 | 4.19 and 5.50 | 2 | 22 | Tart, slightly bitter in aqueous solutions |
| Citric | 192 | 3.14, 4.77 and 6.39 | 3 | 4.3 | Tart, delivers a "burst" of tartness |

total). Tea tannins are frequently mentioned, but tannins amounts are in very low concentrations (catechins being wrongly included under the term "tannin"). As a matter of fact, proanthocyanidins (or condensed tannins) are catechins polymers and are present in very low amounts in green tea and are mainly dimers (trimers are even rarer) (Fraser et al., 2012). Chemical structures of these compounds are detailed in Figure 3.

Flavan-3-ol, including catechins and proanthocyanidins, possess bitter and astringent sensory properties (Fontoin, Saucier, Teissedre, & Glories, 2008; Kielhorn & Thorngate III, 1999; Lesschaeve & Noble, 2005; Peleg, Gacon, Schlich, & Noble, 1999) that may contribute to the mouthfeel of kombucha. The fate of polyphenols during kombucha elaboration still remains enigmatic. Jayabalan et al. (2007) reported a general decrease of epicatechin, epigallocatechin, and their gallate derivatives during the first 9 days of elaboration and then an increase of the nongallated species until the 12th day, suggesting the hydrolysis of the ester bound. Based on the work of Zhu, Zhang, Tsang, Huang, and Chen (1997), an acidic hydrolysis is rather unlikely, which is why an enzymatic origin is speculated. However, the consequences of this phenomenon on the taste and mouthfeel of kombucha remain unknown. A new study (Cardoso et al., 2020) using UPLC-MS (ultra-performance liquid chromatography – mass spectrometry) reported a significant change of phenolic profiles between black tea infusion and kombucha resulting from it. The diversity of compounds increased (27 new compounds), whereas the abundance of compounds decreased. Nevertheless, the global profile did not change drastically with flavonoids remaining the main phenolic compounds followed by phenolic acids. New compounds produced during elaboration mainly belonged to the class of flavonoids. No significant change was observed for green tea and the kombucha made from it. This suggests that kombucha elaboration could affect the phenolic profile, especially for black tea, but would not change it drastically. The initial phenolic profile obtained from infusion is therefore a defining step for the final product regarding this class of compounds.

## 3.4 | The touch: the booze and the fizz

To our knowledge, despite the presence of astringent polyphenols, kombucha is never described as astringent. This is probably due to the presence of sugars that inhibit the perception of astringency, as reported by Lyman and Green (1990).

The perception of ethanol and its influence on other perceptions have been intensively studied to elucidate its impact on the quality of alcoholic beverages. The perception of ethanol occurs across gustatory, olfactory, and trigeminal (or irritation) systems (Cometto-Muñiz & Cain, 1990; Greenwalt et al., 2000; Laska, Distel, & Hudson, 1997). Several studies have reported that the olfaction and nasal irritation thresholds of ethanol ranged around 0.01% (v/v) in water or below, with the irritation (also referred to as "trigeminal") threshold being always higher (Cometto-Muñiz & Cain, 1990; Martin & Pangborn, 1970; Mattes & DiMeglio, 2001). The taste threshold of ethanol ranges around 1 to 2%. This means that ethanol, even if not identifiable on the olfactory level, can impact the aromatic profile of kombucha. On the other hand, the taste of a regular kombucha, with alcohol content below 1%, should not induce a perception of alcohol taste. At near threshold concentration of ethanol, Mattes and DiMeglio (2001) obtained a predominant description of ethanol taste as bitter. Therefore, the crossing of this threshold could impact the perception of kombucha for some consumers and potentially decrease the global appeal of the product, as it can be that case after commercialization on the shelves of retailer (Talebi et al., 2017).

Carbonation affects visual, taste, and trigeminal components. The characteristic oral perception of carbonated drinks is the irritation or trigeminal sensation of tingling (Dessirier, Simons, Carstens, O'Mahony, & Carstens, 2000). Although few investigations have been carried out, the results indicate an enhancement of sourness by carbonation (Cometto-Muniz, Garcia-Medina, & Calvifio, 1987; Yau & McDaniel, 1992). In addition, carbonation has been shown to enhance the perception of cold and *vice versa* (Green, 1992). However, no interaction seems to occur between sweetness and carbonation (Odake, 2001).

## 3.5 | Perceptual interactions

As seen for ethanol and carbon dioxide, chemosensory stimuli do not work independently on perceptions but are involved in interactions even below their own thresholds (Dalton, Doolittle, Nagata, & Breslin, 2000). It has been shown that the visual aspect of beverages influenced significantly both olfactory and taste perception in noncarbonated aqueous solutions (DuBose & Cardello, 1980; Stillman, 1993). The amplification of fruitiness by sourness (and to a lesser extent sweetness) has been reported as a taste–smell interaction. On the contrary, suppressive effects of sourness on sweetness have also been determined as taste–taste interactions (Bonnans & Noble, 1993; Nahon, Roozen, & De Graaf, 1996).

Finally, temperature is a key parameter that impacts the physical chemistry of food and beverages and in particular the volatility of molecules. Kombucha is usually consumed at cold temperature (around 4 °C), as it is commonly marketed and perceived by the consumer as a soft drink. Consequently, sensory evaluation of kombucha should then be assessed at the appropriate temperature.

## 3.6 | What makes kombucha "refreshing"?

Even though the quality components of kombucha have not been defined, one of the most frequently used descriptor for this beverage is the adjective "refreshing" (Dufresne & Farnworth, 2000; Jayabalan et al., 2014; Reiss, 1994; Villarreal-Soto et al., 2018). The real temperature of the food product plays indeed a major role in the perception of "freshness" (Guinard, Souchard, Picot, Rogeaux, & Sieffermann, 1998; Labbe, Gilbert, Antille, & Martin, 2009; Zellner & Durlach, 2002) but it is not the only one. Several studies attempted to define the different components of "freshness." Among them, low viscosity or thickness was judged more "refreshing" in liquids than gels (Labbe et al., 2009; McEwan & Colwill, 1996; Zellner & Durlach, 2002). We can cite also some aroma such as mint, citrus, or peach (Labbe et al., 2009), acidity and low sweetness (Labbe et al., 2009; McEwan & Colwill, 1996). These elements echo the sweetness/sourness balance that has been described as being "basic typical taste profile of all flavored soft drinks." "Without this sweetener and acid balance, the beverage would taste totally wishy-washy and unexciting" (Shachman, 2005).

Kombucha possesses all the characteristics of a refreshing beverage: it is served cold, it is carbonated, sour with low sweetness and viscosity, and exhibits fruity aroma. The identity of a defined product can be modulated by working on different parameters of kombucha's production process ranging from:

- the initial formulation of the sweetened tea;
- the elaboration conditions;
- to bottling and stabilization steps if applicable.

These aspects are discussed in details in the next section of this review.

## 4 | PROCESS PARAMETERS IMPACTING THE COMPOSITION AND QUALITY OF KOMBUCHA

The production of kombucha has been mainly studied by focusing on the elaboration kinetics and the influence of different conditions ranging from the choice of the substrate, the temperature, the inoculum, and even the vessel geometry (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). This section proposes to expand this approach by adding elements relative to the production of the tea liquor.

### 4.1 | Parameters impacting the quality of the tea liquor

The initial composition of water plays a role in the final composition of the tea liquor: the medium in which microorganisms of kombucha grow and exert their activities. Indeed, the mineral and organic composition of water is variable from a region to the other and associated with its hardness, dependent on the calcium and magnesium contents. It was demonstrated that the calcium concentration in water is an important parameter of mineral and organic compounds of tea leaves (Mossion, Potin-Gautier, Delerue, Le Hécho, & Behra, 2008; Spiro & Price, 1987; Spiro, Price, Miller, & Arami, 1987) along with the time/temperature conditions of the infusion (Price & Spitzer, 1993; Price & Spitzer, 1994; Spiro, Jaganyi, & Broom, 1992).

The minerals had different behaviors during the extraction depending on the ions when using distilled water (Matsuura, Hokura, Katsuki, Itoh, & Haraguchi, 2001; Ødegård & Lund, 1997):

- Sodium, potassium, and nickel are strongly extracted (>55%);
- Magnesium, aluminum, manganese, and zinc are moderately extracted (between 20 and 55%);
- Calcium, iron, and copper are poorly extracted (<20%).

When mineral water is used, the ions with the lowest concentrations show variation of their extraction yield of 10% maximum according to Mossion (2007) (approximately several mg/L). The nature of the tea induces a strong variability in extraction behaviors.

Calcium possesses a particular behavior, especially if the infusion water possesses a significant amount of it as it is the case for mineral water: an inversed flux was observed from

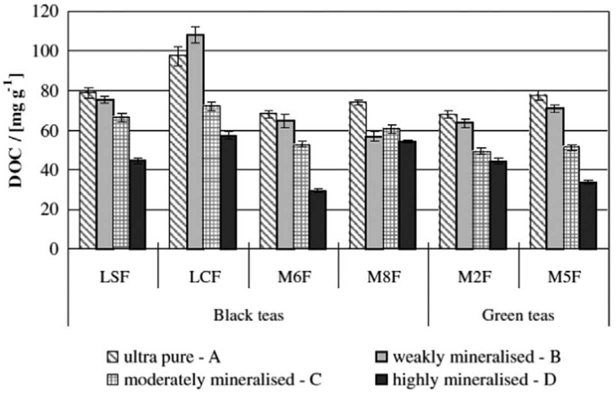

**FIGURE 4** Effect of water composition on dissolved organic carbon content extracted from tea leaves per gram of brewed leaves (mg C/g) (Mossion et al., 2008)

water to the tea leaves (Anderson, Hollins, & Bond, 1971; Mossion, 2007; Mossion et al., 2008). It is speculated that pectins present in the cell walls of the tea cells bind to $Ca^{2+}$ ions (Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006; Spiro et al., 1987), inducing structure modifications that inhibit the extraction of compounds (Figure 4 [Mossion et al., 2008]).

It has also been shown that the extraction of organic matter was enhanced by:

- the increase in temperature (ranging above 70 °C) and infusion duration (between 1 min and 1 hr);
- a weaker mineral content;
- more specifically, a lower calcium content (corresponding to lower water hardness).

In conclusion, temperature, calcium content of water, and the nature of the tea are the main parameters involved in the making of the tea liquor. This matrix will affect the growth and activities of the kombucha cultures' microorganisms.

## 4.2 | Impact of the nature of tea on microbial dynamics

According to Kallel et al. (2012), a consumption of sucrose, glucose, fructose, and the production of organic acids and cellulose are faster and more intense in black tea than in green tea in identical elaboration conditions. This was not observed in other studies, in which no impact on the physical chemistry could be observed (Jayabalan et al., 2007) or gave the opposite effect (Coton et al., 2017). On the contrary, at the microbiological level, it was reported that the nature of tea did not impact the dynamics of yeasts but the use of green tea allowed the development of *Oenococcus oeni* that was absent in the black tea modality (Figure 5 [Coton et al., 2017]). In the same study, a higher bacterial biodiversity could be observed in green tea than in black tea, in which domination phenomenon is more present in the liquid phase. In the biofilm, the domination of *Gluconobacter* is effective in all modalities after 2 days of elaboration to the detriment of *O. oeni*.

## 4.3 | Impact of the carbohydrate substrate and of its initial amount

The use of different carbohydrates as substrate has been investigated by Reiss (1994). Sucrose is hydrolyzed by yeasts into glucose and fructose. In this study, glucose favors the production of lactic acid and fructose the production of ethanol. Moreover, maltose has been poorly consumed and lactose did not affect the yield of ethanol nor stimulated the production of lactic acid. Acetic acid bacteria transform glucose in gluconic acid and fructose in acetic acid. Beside carbohydrates, it was also reported that lactic acid enhanced the production of biomass.

The utilization of molasses with 50% sucrose content (wet weight) was studied at different rates (35, 50, and 70% of total batch volume) (Malbaša et al., 2008; Malbaša, Lončar, Djurić, & Došenović, 2008). With 35 and 50 g/L of molasses, kinetics of sucrose consumption, pH variation, and production of organic acids were similar; whereas with 70 g/L of molasses, the consumption of sucrose was much faster and the production of lactic acid was enhanced to the detriment of acetic acid but with identical total acidity values. Moreover, the decrease in pH was less intense, probably due to the buffering capacity of molasses.

The increase of the initial content in sucrose led to the increase of the production of cellulose until a limit concentration (90 g/L) after which a decrease of cellulose production could be observed. The increase of initial sucrose concentration between 70 and 110 g/L accelerates the decrease of pH following a dose effect. The growth of yeast and bacterial populations was also stimulated by the increase of initial sucrose concentration (Goh et al., 2012a). A similar trend was observed in the work of Blanc (1996) but to a lesser extent.

## 4.4 | The inoculum

As stated in Section 2, several methods are reported: using a previous batch of kombucha, only the broth can be added (Blanc, 1996; Jayabalan et al., 2007; Loncar et al., 2014; Malbaša et al., 2008), only the biofilm or fragments (Jayabalan et al., 2010; Reiss, 1994; Sievers et al., 1995), or both (Chen & Liu, 2000; Goh et al., 2012a; Kallel et al., 2012)

### 4.4.1 | Nature of the inoculum

Several studies have compared elaboration kinetics with different kombucha inocula. Chu and Chen (2006) observed different kinetics of antioxidant capacities and total phenolic content increases over eight differently sourced kombucha cultures used as inocula.

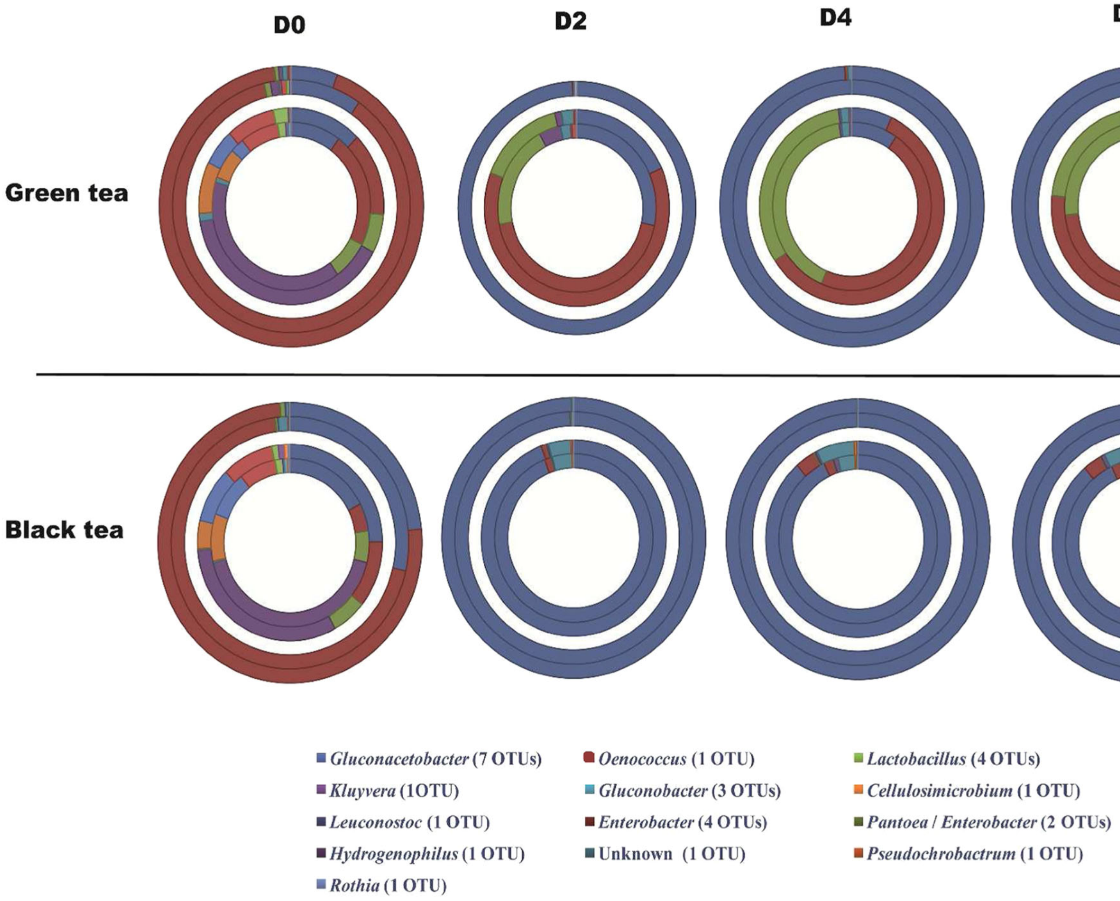

**FIGURE 5** Relative abundance of bacterial species in green and black tea Kombucha elaboration based on 16S rDNA metabarcoding. The 28 most abundant OTUs (Operational Taxonomic Units) out of total 354 OTUs are presented on this figure (>1% relative abundance). Sampling was performed at days 0, 2, 4, and 8 on both tea (two inner circles) and biofilm (two outer circles) samples. The two circles represent replicate samples for each sample type (Coton et al., 2017).

The elaboration kinetics of a kombucha inoculum and two controlled inocula prepared from kombucha isolates (a mix of acetic acid bacteria and one yeast: *Zygosaccharomyces* sp. and *Saccharomyces cerevisiae*, respectively) were compared (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011). Similar pH variations in black tea and green tea matrices were observed but the production of C and B vitamins showed significant differences with the original consortium producing always the highest amount of vitamin B. The highest amount of vitamin C was produced by the original consortium and the controlled consortium including *S. cerevisiae*. Another study using cocultures from isolated microorganisms (*Gluconobacter intermedius* coinoculated with *Dekkera bruxellensis*) was carried out in order to optimize the production of health beneficial glucuronic acid by playing with the relative proportion of each strain with significant results (Nguyen et al., 2014). Currently, no link has been established between the presence of particular genera or species and general chemical profiles of kombucha.

### 4.4.2 | Amount of inoculum used

The addition of 15% inoculum (unknown chemical and microbiological composition "from previous batch") compared to 10% demonstrated a consumption of sucrose, glucose, fructose and acidification faster at a given temperature (22 °C or 30 °C) on the first 10 days of elaboration (Figure 6 [Lončar et al., 2006]). This suggests that increasing the quantity of inoculum accelerates the elaboration kinetics (Lončar et al., 2006).

## 4.5 | The temperature

In the study of Lončar et al. (2006, 2014), temperature was determined to be a parameter more impactful on elaboration kinetics compared to the amount of added inoculum. When 22 °C and 30 °C elaboration temperatures were compared, the consumption of sugars and the acidification appeared to be faster at 30 °C for a given quantity of added inoculum (10% and 15%) on the first 10 days. Conclusion is that higher

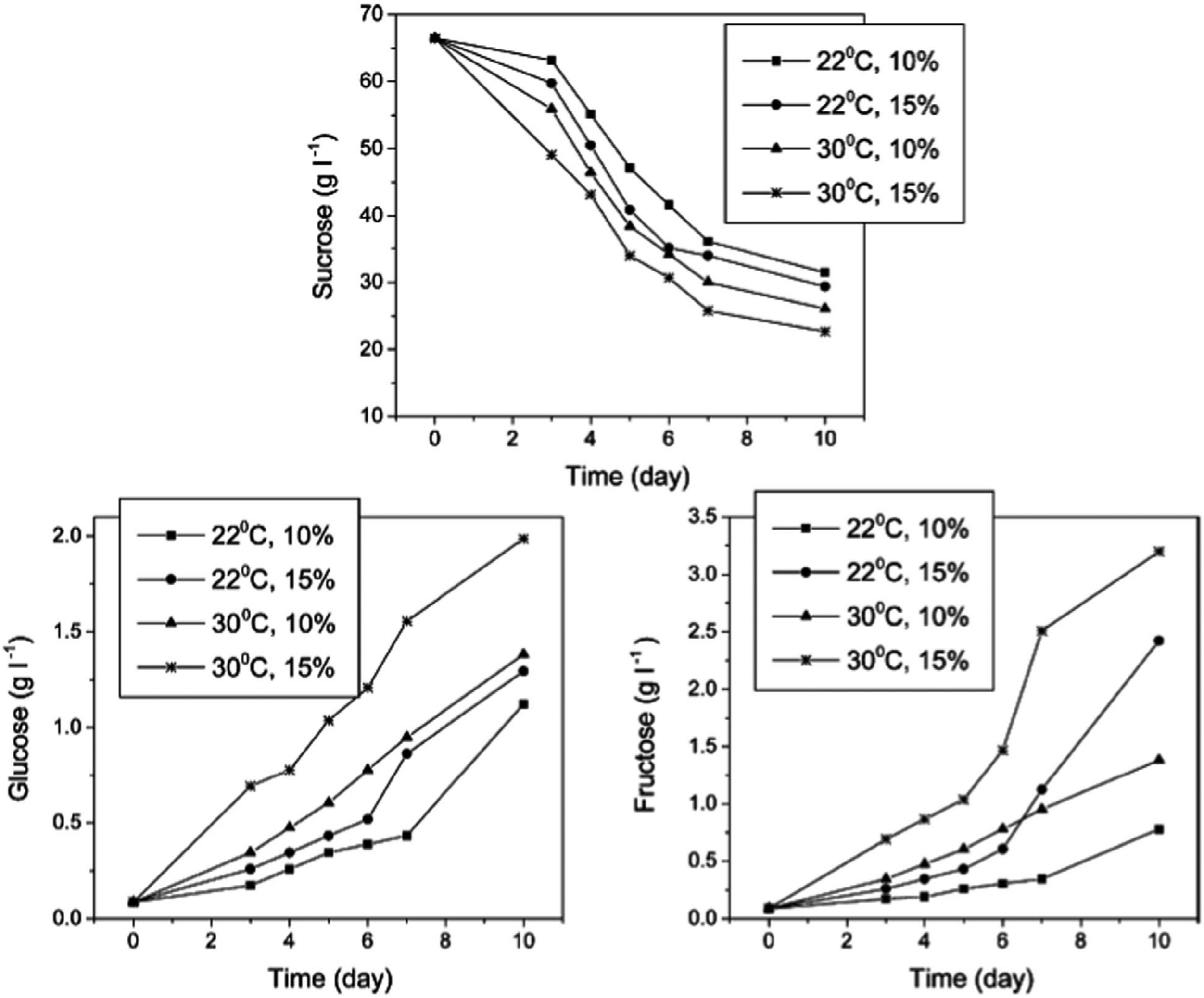

**FIGURE 6** Sugar quantities as functions of time, temperature, and inoculum concentration (Lončar et al., 2006)

**TABLE 3** Changes in total count of acetic acid bacteria, yeast, lactic acid bacteria, and pH values in Kombucha beverages during 10 days of elaboration at 20, 25, and 30 °C (adapted from Neffe-Skocińska et al. [2017])

| Microbial species (log CFU/mL)/pH | Temperature of elaboration (°C) | Days of elaboration | | | |
|---|---|---|---|---|---|
| | | 0 | 3 | 7 | 10 |
| AAB | 20 | 3.57 ± 0.2$^{aA}$ | 4.60 ± 0.3$^{aB}$ | 6.72 ± 0.1$^{aC}$ | 7.39 ± 0.1$^{bD}$ |
| | 25 | 3.93 ± 0.1$^{aA}$ | 4.90 ± 0.2$^{aB}$ | 6.90 ± 0.2$^{aC}$ | 7.61 ± 0.2$^{bD}$ |
| | 30 | 3.65 ± 0.2$^{aA}$ | 5.15 ± 0.1$^{aB}$ | 7.10 ± 0.2$^{aC}$ | 6.77 ± 0.2$^{aC}$ |
| Yeast | 20 | 4.02 ± 0.1$^{aA}$ | 5.86 ± 0.2$^{bB}$ | 7.00 ± 0.1$^{bC}$ | 7.83 ± 0.2$^{cD}$ |
| | 25 | 4.24 ± 0.1$^{aA}$ | 7.00 ± 0.2$^{cB}$ | 7.22 ± 0.1$^{bB}$ | 7.43 ± 0.1$^{bB}$ |
| | 30 | 4.01 ± 0.2$^{aA}$ | 4.73 ± 0.1$^{aB}$ | 5.48 ± 0.1$^{aC}$ | 36.40 ± 0.2$^{aD}$ |
| pH | 20 | 3.08 ± 0.1$^{aA}$ | 2.85 ± 0.1$^{aA}$ | 2.88 ± 0.1$^{aA}$ | 2.67 ± 0.1$^{aA}$ |
| | 25 | 3.07 ± 0.1$^{aB}$ | 2.80 ± 0.1$^{aA}$ | 2.79 ± 0.1$^{aA}$ | 2.77 ± 0.2$^{aA}$ |
| | 30 | 3.04 ± 0.1$^{aB}$ | 2.81 ± 0.1$^{aA}$ | 2.71 ± 0.1$^{aA}$ | 2.63 ± 0.2$^{aA}$ |

Data are expressed as mean ± standard deviation of $n = 3$ samples.
Means in the same column followed by different lowercase letters represent significant differences ($P < 0.05$).
Means in the same row followed by different uppercase letters represent significant differences ($P < 0.05$).

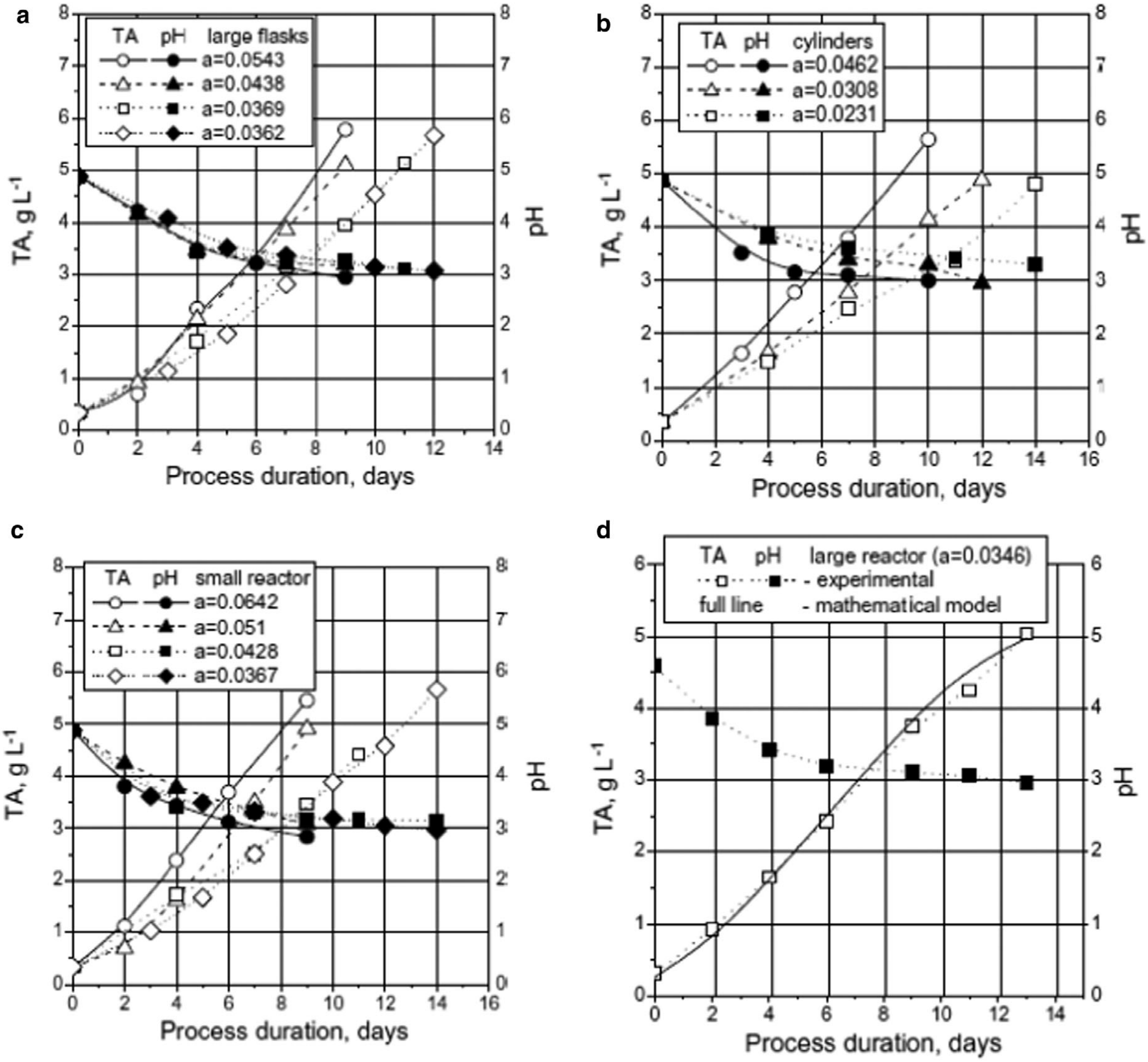

**FIGURE 7** Changes in pH value and total acidity of kombucha produced in flasks (a), cylinders (b), small reactors (c), and large reactors (d) (adapted from Cvetković et al. [2008])

temperature also accelerates the elaboration kinetics. This is contradictory with the results of Neffe-Skocińska et al. (2017) who evaluated an optimal temperature of 25 °C because it could favor the yeasts' activity whose metabolites (monosaccharides and ethanol) are the only available substrates for acetic acid bacteria at the beginning of the process (Table 3). Thus, the growth and metabolic activity of yeasts in sucrose-based kombucha is the limiting step of the process. Yet, no significant difference in sensory profile could be determined between the kombucha produced at 20, 25, and 30°C.

Another study (De Filippis et al., 2018) reported that a temperature of 30 °C compared to 20 °C increased the population of acetic acid bacteria. Each modality operated a selection of bacteria species: *Gluconobacter saccharivorans* was in majority at 30 °C to the detriment of *G. xylinus*, which was dominant at 20 °C. At 30 °C, the enhancement of *G. saccharivorans* population also enhanced the production of gluconic and glucuronic acids.

## 4.6 | The geometry of the vessel

According to Malbaša et al. (2006), the geometry of the elaboration tank or vessel was a parameter more impactful than the amount of added inoculum. In the same study, the parameter of geometric similarity for a cylindrical vessel was highlighted and defined as

$$\frac{D_{T2}}{D_{T1}} = \left(\frac{V_{T2}}{V_{T1}}\right)^{1/3}$$

$D_{Tx}$ is defined as the diameter of the cylinder $x$ and $V_{Tx}$ as the volume of the cylinder $x$. The elaboration kinetics seems to be characterized by a relationship between the vessel's diameter and volume.

Another parameter of the same type was investigated: the specific interfacial surface (SIS) defined as

$$\textit{Specific Interfacial Surface}\ \left(cm^{-1}\right) = \frac{\textit{Surface of liquid}\ \left(cm^{2}\right)}{\textit{Volume of liquid}\ \left(cm^{3}\right)}$$

The accuracy of this model was tested with different geometries on 56 vessels from 90 L reactors to 330 mL flasks and could be validated (Cvetković, Markov, Djurić, Savić, & Velićanski, 2008). This model was judged to be more efficient than the geometric similarity because the study showed a higher fidelity of elaboration kinetics and durations to the SIS mathematic model (Figure 7) (Cvetković et al., 2008; Malbaša et al., 2006). The study shows that regardless of the shape of the vessel (flask, cylinder, or reactor) and the size, a larger SIS induced faster acidification kinetics. The increase of SIS can be achieved by using a larger air/liquid interface and/or by reducing the volume of liquid. Consequence results in better conditions for oxygen access for acetic acid bacteria located in the biofilm or the liquid phase. A smart use of the SIS parameter can help controlling the speed of the acidification phase of kombucha elaboration.

As a conclusion, ways to increase the speed of the acidification phase of kombucha elaboration is to increase the amount of inoculum (ranging between 10% and 15%), increase the temperature up to around 30 °C, and to maximize the SIS. The water composition, tea type, and the choice of substrates interdependently influence composition of the initial matrix. Thus, these parameters need to be assessed on a case-by-case-basis depending on the targeted result. Eventually, the influence of sugared tea liquor and microbial composition and the interaction of these both elements on the final product remain shrouded in mystery and there is a need for research to be carried out on these problematics.

## 5 | CONCLUSION AND PERSPECTIVES

Kombucha can be approached the same way as a carbonated soft drink: emphasis is put on its visual aspect, the aroma profile, and the taste, in particular the sweetness/sourness balance. Yet, due to the infinity of combination of microbial compositions, the multiple processes used at home and in the industry and the lack of specific quality standards in regulation, the characterization of kombucha's quality dimension remains a challenge (Watson, 2019). If the authenticity of kombucha had to be defined by the expectations of the consumer (Monaco, 2019), it would need to take the following elements into account:

- The rawness of the product, namely the preservation of potential beneficial properties;
- The stability of the product over time;
- The accessibility of the product by offering a pleasant sensory profile from appearance to taste.

Therefore, decisions need to be taken about the microbial and chemical stabilization of the product and the production process needs to be adapted accordingly. Consequently, interdependent parameters during the production process will impact the consumer perception of the final product. The knowledge of such complex systems is still lacking as producers and scientists face many grey areas.

The process of kombucha production was mainly studied by the determination of the microbial composition and the understanding of the main technological roles attributed to yeasts and bacteria through the consumption of carbohydrates and the production of organic acids. The impact of environmental factors, such as temperature, the substrate content, and the vessel geometry, was investigated. A characterization of kombucha consortia composition involving a large number of samples at worldwide scale would help define kombucha better and highlight signature genera, species, and co-occurrences between microorganisms. The existence of an effect of the geographical sourcing on the microbial composition could even lead to the concept of kombucha typicity. As a matter of fact, few studies have investigated the microbial dynamic and tried to explain the reasons why certain genera or species were dominant in given conditions during elaboration (Coton et al., 2017; De Filippis et al., 2018; Teoh et al., 2004). Moreover, the intraspecific diversity was not investigated to our knowledge. Indeed, the questions of the origin of kombucha consortia and their stability or evolution over time, namely in relationship to the matrix composition (tea, substrate) and the process conditions, still remain unanswered. Nevertheless, the available knowledge can already allow the orientations of the microbial activity and the kinetics of elaboration. Further control or prediction on kombucha elaboration remains difficult when it comes to the organoleptic profile.

Surprisingly, little data are available about the sensory of kombucha *per se* and namely the compounds involved in its aroma profile. To our knowledge, no research was carried out to draw lines between the odorant volatile molecules and the microbial composition. As a matter of fact, beside the main metabolites such as organic acids, ethanol and the substances of interest such as vitamins, gluconic, glucuronic, or D-saccharic-1,4-lactone acids, little is known of the metabolic activity of the consortium. Without a doubt, microbial interactions are occurring during the elaboration (Teoh et al., 2004), first through the symbiosis between yeasts and acetic acid bacteria and possibly through nutrient competition or/and targeted chemical signals such as peptides or messenger RNAs (Ivey, Massel, & Phister, 2013; Leroi & Pidoux, 1993; Sieuwerts, Bron, & Smid, 2018; C. Wang, Mas, & Esteve-Zarzoso, 2016). The utilization of "omics" techniques such as metabolomics or transcriptomics, which are nontargeted analyses of metabolites and gene expression, respectively, could open doors in the study of microbial interactions in products as it is the case for wine (Liu et al., 2016). Kombucha is seen as a promising model system for the study of microbial interaction in symbiotic systems (May et al., 2019). The elucidation of such interactions could give way to better control of elaboration processes in terms of timing and repeatability, and thus spark significant interest in the dynamic kombucha community of producers and consumers.

## ACKNOWLEDGMENTS

This work was supported by the Regional Council of Bourgogne – Franche Comté, the "Fond Européen de DEveloppement Régional (FEDER). Authors declare no conflict of interest. We thank Jordi Ballester for his precious feedbacks on the sensory aspects of the review.

## AUTHOR CONTRIBUTIONS

Thierry Tran took the lead of the writing of this review but all other authors provided critical and complementary elements to the manuscript.

## ORCID

*Thierry Tran* https://orcid.org/0000-0002-5666-3829

## REFERENCES

Acree, T. E., & van Ruth, S. (2003). Gas chromatography/olfactometry. *Current Protocols in Food Analytical Chemistry*, *10*(1), G1.8.1–G1.8.12. https://10.1002/0471142913.fag0108s10

Alhede, M., Kragh, K. N., Qvortrup, K., Allesen-Holm, M., van Gennip, M., Christensen, L. D., … Bjarnsholt, T. (2011). Phenotypes of non-attached *pseudomonas aeruginosa* aggregates resemble surface attached biofilm. *PLoS ONE*, *6*(11), e27943. https://doi.org/10.1371/journal.pone.0027943

Ali, S. A., & Shivanna, G. B. (2017). Physico-chemical characteristics of tea fungus: Kombucha. *International Journal of Pharmacy and Pharmaceutical Sciences*, *9*(4), 161. https://doi.org/10.22159/ijpps.2017v9i4.16753

Anderson, W., Hollins, J. G., & Bond, P. S. (1971). The composition of tea infusions examined in relation to the association between mortality and water hardness. *Journal of Hygiene*, *69*(1), 1–15. https://doi.org/10.1017/S0022172400021197

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. M. (1997). The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition*, *37*(8), 693–704. https://doi.org/10.1080/10408399709527797

Barker, G. S., Jefferson, B., & Judd, S. J. (2002). The control of bubble size in carbonated beverages. *Chemical Engineering Science*, *57*(4), 565–573. https://doi.org/10.1016/S0009-2509(01)00391-8

Battikh, H., Bakhrouf, A., & Ammar, E. (2012). Antimicrobial effect of Kombucha analogues. *LWT - Food Science and Technology*, *47*(1), 71–77. https://doi.org/10.1016/j.lwt.2011.12.033

Bauer-Petrovska, B., & Petrushevska-Tozi, L. (2000). Mineral and water soluble vitamin content in the Kombucha drink. *International Journal of Food Science & Technology*, *35*(2), 201–205.

Bhattacharya, S., Gachhui, R., & Sil, P. C. (2013). Effect of Kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. *Food and Chemical Toxicology*, *60*, 328–340. https://doi.org/10.1016/j.fct.2013.07.051

Blanc, P. J. (1996). Characterization of the tea fungus metabolites. *Biotechnology Letters*, *18*(2), 139–142. https://doi.org/10.1007/BF00128667

Bonnans, S., & Noble, A. C. (1993). Effect of sweetener type and of sweetener and acid levels on temporal perception of sweetness, sourness and fruitiness. *Chemical Senses*, *18*(3), 273–283. https://doi.org/10.1093/chemse/18.3.273

Capel, F., Nicolai, T., Durand, D., Boulenguer, P., & Langendorff, V. (2006). Calcium and acid induced gelation of (amidated) low methoxyl pectin. *Food Hydrocolloids*, *20*(6), 901–907. https://doi.org/10.1016/j.foodhyd.2005.09.004

Cardoso, R. R., Neto, R. O., dos Santos D'Almeida, C. T., do Nascimento, T. P., Pressete, C. G., Azevedo, L., … de Barros, F. A. R. (2020). Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International*, *128*, 108782. https://doi.org/10.1016/j.foodres.2019.108782

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology*, *220*, 63–72. https://doi.org/10.1016/j.ijfoodmicro.2015.12.015

Charters, S., & Pettigrew, S. (2007). The dimensions of wine quality. *Food Quality and Preference*, *18*(7), 997–1007. https://doi.org/10.1016/j.foodqual.2007.04.003

Chen, C., & Liu, B. Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology*, *89*(5), 834–839.

Chu, S.-C., & Chen, C. (2006). Effects of origins and fermentation time on the antioxidant activities of Kombucha. *Food Chemistry*, *98*(3), 502–507. https://doi.org/10.1016/j.foodchem.2005.05.080

Cometto-Muñiz, J. E., & Cain, W. S. (1990). Thresholds for odor and nasal pungency. *Physiology & Behavior*, *48*(5), 719–725. https://doi.org/10.1016/0031-9384(90)90217-R

Cometto-Muniz, J. E., Garcia-Medina, M. R., & Calvifio, A. M. (1987). Interactions between $CO_2$ oral pungency and taste. *Perception*, *16*(5), 629–640.

Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme-Labarthe, L., … Coton, E. (2017). Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. *FEMS Microbiology Ecology*, *93*(5). https://doi.org/10.1093/femsec/fix048

Cvetković, D., Markov, S., Djurić, M., Savić, D., & Velićanski, A. (2008). Specific interfacial area as a key variable in scaling-up

Kombucha fermentation. *Journal of Food Engineering*, *85*(3), 387–392. https://doi.org/10.1016/j.jfoodeng.2007.07.021

Da Conceicao Neta, E. R., Johanningsmeier, S. D., & McFeeters, R. F. (2007). The chemistry and physiology of sour taste? a review. *Journal of Food Science*, *72*(2), R33–R38. https://doi.org/10.1111/j.1750-3841.2007.00282.x

Dalton, P., Doolittle, N., Nagata, H., & Breslin, P. A. S. (2000). The merging of the senses: Integration of subthreshold taste and smell. *Nature Neuroscience*, *3*(5), 431–432.

David-Vaizant, V., & Alexandre, H. (2018). Flor yeast diversity and dynamics in biologically aged wines. *Frontiers in Microbiology*, *9*, 2235. https://doi.org/10.3389/fmicb.2018.02235

De Filippis, F., Troise, A. D., Vitaglione, P., & Ercolini, D. (2018). Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. *Food Microbiology*, *73*, 11–16. https://doi.org/10.1016/j.fm.2018.01.008

De Ley, J. (1961). Comparative carbohydrate metabolism and a proposal for a phylogenetic relationship of the acetic acid bacteria. *Microbiology*, *24*(1), 31–50.

Descoins, C., Mathlouthi, M., Le Moual, M., & Hennequin, J. (2006). Carbonation monitoring of beverage in a laboratory scale unit with on-line measurement of dissolved $CO_2$. *Food Chemistry*, *95*(4), 541–553. https://doi.org/10.1016/j.foodchem.2004.11.031

Dessirier, J.-M., Simons, C. T., Carstens, M. I., O'Mahony, M., & Carstens, E. (2000). Psychophysical and neurobiological evidence that the oral sensation elicited by carbonated water is of chemogenic origin. *Chemical Senses*, *25*(3), 277–284. https://doi.org/10.1093/chemse/25.3.277

DuBose, N., & Cardello, V. (1980). Effects Of colorants and flavorants on identification, percieved flavor intensity, and hedonic quality of fruit-flavored beverages and cake. *Journal of Food Science*, *45*(5), 1393–1399.

Dufresne, C., & Farnworth, E. (2000). Tea, kombucha, and health : A review. *Food Research International*, *33*(6), 409–421. https://doi.org/10.1016/S0963-9969(00)00067-3

Emiljanowicz, K. E., & Malinowska-Pańczyk, E. (2019). Kombucha from alternative raw materials – The review. *Critical Reviews in Food Science and Nutrition*, *10*, 1–10. https://doi.org/10.1080/10408398.2019.1679714

Ernst, E. (2003). Kombucha : A systematic review of the clinical evidence. *Complementary Medicine Research*, *10*(2), 85–87.

Fia, G., Giovani, G., & Rosi, I. (2005). Study of beta-glucosidase production by wine-related yeasts during alcoholic fermentation. A new rapid fluorimetric method to determine enzymatic activity. *Journal of Applied Microbiology*, *99*(3), 509–517. https://doi.org/10.1111/j.1365-2672.2005.02657.x

Fontoin, H., Saucier, C., Teissedre, P.-L., & Glories, Y. (2008). Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference*, *19*(3), 286–291. https://doi.org/10.1016/j.foodqual.2007.08.004

Fraser, K., Harrison, S. J., Lane, G. A., Otter, D. E., Hemar, Y., Quek, S.-Y., & Rasmussen, S. (2012). HPLC–MS/MS profiling of proanthocyanidins in teas : A comparative study. *Journal of Food Composition and Analysis*, *26*(1–2), 43–51. https://doi.org/10.1016/j.jfca.2012.01.004

Gamboa-Gómez, C. I., González-Laredo, R. F., Gallegos-Infante, J. A., Larrosa Pérez, M., del, M., Moreno-Jiménez, M. R., … Rocha-Guzmán, N. E. (2016). Antioxidant and angiotensin-converting enzyme inhibitory activity of *eucalyptus camaldulensis* and *litsea glaucescens* infusions fermented with kombucha consortium. *Food Technology and Biotechnology*, *54*(3), 367–374. https://doi.org/10.17113/ftb.54.03.16.4622

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012a). Fermentation of black tea broth (Kombucha) : I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*, *19*(1), 109–117.

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012b). Microstructure and physical properties of microbial cellulose produced during fermentation of black tea broth (Kombucha). II. *International Food Research Journal*, *19*(1), 153–158.

Green, B. G. (1992). The effects of temperature and concentration on the perceived intensity and quality of carbonation. *Chemical Senses*, *17*(5), 435–450.

Greenwalt, C. J., Steinkraus, K. H., & Ledford, R. A. (2000). Kombucha, the fermented tea : Microbiology, composition, and claimed health effects. *Journal of Food Protection*, *63*(7), 976–981.

Guinard, J.-X., Souchard, A., Picot, M., Rogeaux, M., & Sieffermann, J.-M. (1998). Sensory determinants of the thirst-quenching character of beer. *Appetite*, *31*, 101–115.

Harbowy, M. E., Balentine, D. A., Davies, A. P., & Cai, Y. (1997). Tea chemistry. *Critical Reviews in Plant Sciences*, *16*(5), 415–480. https://doi.org/10.1080/07352689709701956

Harris, N., & Ellis, R. T. (1981). Black tea manufacture. I. Effects on leaf structure of different processing systems. *Annals of Applied Biology*, *99*(3), 359–366. https://doi.org/10.1111/j.1744-7348.1981.tb04805.x

Haslam, E. (2003). Thoughts on thearubigins. *Phytochemistry*, *64*(1), 61–73. https://doi.org/10.1016/S0031-9422(03)00355-8

Hernandez, L. F., Espinosa, J. C., Fernandez-Gonzalez, M., & Briones, A. (2003). $\beta$-Glucosidase activity in a *Saccharomyces cerevisiae* wine strain. *International Journal of Food Microbiology*, *80*(2), 171–179.

Ho, C.-T., Zheng, X., & Li, S. (2015). Tea aroma formation. *Food Science and Human Wellness*, *4*(1), 9–27. https://doi.org/10.1016/j.fshw.2015.04.001

Holbourn, A., & Hurdman, J. (2017). Kombucha : Is a cup of tea good for you? *BMJ Case Reports*, https://doi.org/10.1136/bcr-2017-221702

Ivanišová, E., Meňhartová, K., Terentjeva, M., Harangozo, Ľ., Kántor, A., & Kačániová, M. (2019). The evaluation of chemical, antioxidant, antimicrobial and sensory properties of kombucha tea beverage. *Journal of Food Science and Technology*, *57*, 1840–1846 https://doi.org/10.1007/s13197-019-04217-3

Ivey, M., Massel, M., & Phister, T. G. (2013). Microbial interactions in food fermentations. *Annual Review of Food Science and Technology*, *4*(1), 141–162. https://doi.org/10.1146/annurev-food-022811-101219

Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chemistry*, *102*(1), 392–398. https://doi.org/10.1016/j.foodchem.2006.05.032

Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., & Swaminathan, K. (2008). Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry*, *109*(1), 227–234. https://doi.org/10.1016/j.foodchem.2007.12.037

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538–550. https://doi.org/10.1111/1541-4337.12073

Jayabalan, R., Malini, K., Sathishkumar, M., Swaminathan, K., & Yun, S.-E. (2010). Biochemical characteristics of tea fungus produced during kombucha fermentation. *Food Science and Biotechnology*, *19*(3), 843–847. https://doi.org/10.1007/s10068-010-0119-6

Jayabalan, R., Marimuthu, S., Thangaraj, P., Sathishkumar, M., Binupriya, A. R., Swaminathan, K., & Yun, S. E. (2008). Preservation of kombucha tea-effect of temperature on tea components and free radical scavenging properties. *Journal of Agricultural and Food Chemistry*, *56*(19), 9064–9071. https://doi.org/10.1021/jf8020893

Jones, S. F., Evans, G. M., & Galvin, K. P. (1999). The cycle of bubble production from a gas cavity in a supersaturated solution. *Advances in Colloid and Interface Science*, *80*(1), 51–84. https://doi.org/10.1016/S0001-8686(98)00075-X

Kallel, L., Desseaux, V., Hamdi, M., Stocker, P., & Ajandouz, E. H. (2012). Insights into the fermentation biochemistry of Kombucha teas and potential impacts of Kombucha drinking on starch digestion. *Food Research International*, *49*(1), 226–232. https://doi.org/10.1016/j.foodres.2012.08.018

Kappes, S. M., & Schmidt, S. J. (2007). Relationship between physical properties and sensory attributes of carbonated beverages. *Journal of Food Science*, *72*(1), 11.

Khan, N., & Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life Sciences*, *81*(7), 519–533. https://doi.org/10.1016/j.lfs.2007.06.011

Kielhorn, S., & Thorngate, III, J. H. (1999). Oral sensations associated with the flavan–3-ols (+)-catechin and (-)-epicatechin. *Food Quality and Preference*, *10*(2), 109–116.

Kumar, V., & Joshi, V. K. (2016). Kombucha: Technology, microbiology, production, composition and therapeutic value. *International Journal of Food and Fermentation Technology*, *6*(1), 13–24. https://doi.org/10.5958/2277-9396.2016.00022.2

Labbe, D., Gilbert, F., Antille, N., & Martin, N. (2009). Sensory determinants of refreshing. *Food Quality and Preference*, *20*(2), 100–109. https://doi.org/10.1016/j.foodqual.2007.09.001

Laska, M., Distel, H., & Hudson, R. (1997). Trigeminal perception of odorant quality in congenitally anosmic subjects. *Chemical Senses*, *22*(4), 447–456.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International journal of food microbiology*, *55*(1), 181–186.

Leroi, F., & Pidoux, M. (1993). Detection of interactions between yeasts and lactic acid bacteria isolated from sugary kefir grains. *Journal of Applied Bacteriology*, *74*(1), 48–53. https://doi.org/10.1111/j.1365-2672.1993.tb02995.x

Lesschaeve, I., & Noble, A. C. (2005). Polyphenols : Factors influencing their sensory properties and their effects on food and beverage preferences. *The American Journal of Clinical Nutrition*, *81*(1), 330S–335S. https://doi.org/10.1093/ajcn/81.1.330S

Li, H., & Liu, F. (2015). The chemistry of sour taste and the strategy to reduce the sour taste of beer. *Food Chemistry*, *185*, 200–204. https://doi.org/10.1016/j.foodchem.2015.03.135

Lin, S.-P., Loira Calvar, I., Catchmark, J. M., Liu, J.-R., Demirci, A., & Cheng, K.-C. (2013). Biosynthesis, production and applications of bacterial cellulose. *Cellulose*, *20*(5), 2191–2219. https://doi.org/10.1007/s10570-013-9994-3

Liu, Y., Forcisi, S., Harir, M., Deleris-Bou, M., Krieger-Weber, S., Lucio, M., … Alexandre, H. (2016). New molecular evidence of wine yeast-bacteria interaction unraveled by non-targeted exometabolomic profiling. *Metabolomics*, *12*(69). https://doi.org/10.1007/s11306-016-1001-1

Lončar, E., Djurić, M., Malbaša, R., Kolarov, L. J., & Klašnja, M. (2006). Influence of working conditions upon kombucha conducted fermentation of black tea. *Food and Bioproducts Processing*, *84*(3), 186–192. https://doi.org/10.1205/fbp.04306

Loncar, E., Kanuric, K., Malbasa, R., Djuric, M., & Milanovic, S. (2014). Kinetics of saccharose fermentation by Kombucha. *Chemical Industry and Chemical Engineering Quarterly*, *20*(3), 345–352. https://doi.org/10.2298/CICEQ121113016L

Lubetkin, S., & Blackwell, M. (1988). The nucleation of bubbles in supersaturated solutions. *Journal of Colloid and Interface Science*, *126*(2), 610–615. https://doi.org/10.1016/0021-9797(88)90161-0

Lyman, B. J., & Green, B. G. (1990). Oral astringency : Effects of repeated exposure and interactions with sweeteners. *Chemical Senses*, *15*(2), 151–164. https://doi.org/10.1093/chemse/15.2.151

Lynch, K. M., Zannini, E., Wilkinson, S., Daenen, L., & Arendt, E. K. (2019). Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. *Comprehensive Reviews in Food Science and Food Safety*, *18*(3), 587–625. https://doi.org/10.1111/1541-4337.12440

Malbaša, R., Lončar, E., & Djurić, M. (2008). Comparison of the products of Kombucha fermentation on sucrose and molasses. *Food Chemistry*, *106*(3), 1039–1045. https://doi.org/10.1016/j.foodchem.2007.07.020

Malbaša, R., Lončar, E., Djurić, M., & Došenović, I. (2008). Effect of sucrose concentration on the products of Kombucha fermentation on molasses. *Food Chemistry*, *108*(3), 926–932. https://doi.org/10.1016/j.foodchem.2007.11.069

Malbaša, R., Lončar, E., Djurić, M., Klašnja, M., Kolarov, L. J., & Markov, S. (2006). Scale-up of black tea batch fermentation by kombucha. *Food and Bioproducts Processing*, *84*(3), 193–199. https://doi.org/10.1205/fbp.05061

Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Čanadanović-Brunet, J. M. (2011). Influence of starter cultures on the antioxidant activity of kombucha beverage. *Food Chemistry*, *127*(4), 1727–1731. https://doi.org/10.1016/j.foodchem.2011.02.048

Mangas, J. J., González, M. P., Rodríguez, R., & Blanco, D. (1996). Solid-phase extraction and determination of trace aroma and flavour components in cider by GC-MS. *Chromatographia*, *42*(1–2), 101–105. https://doi.org/10.1007/BF02271063

Markov, S., Cvetković, D., & Bukvić, B. (2005). Use of tea fungus isolate as starter culture for obtaining of kombucha. *8th International Symposium Interdisciplinary Regional Research Hungary-Romania-Serbia and Montenegro*, Szeged, Hungary 19–21.

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology*, *38*, 171–178. https://doi.org/10.1016/j.fm.2013.09.003

Martin, S., & Pangborn, R. M. (1970). Taste interaction of ethyl alcohol with sweet, salty, sour and bitter compounds. *Journal of the Science of Food and Agriculture*, *21*(12), 653–655.

Martínez Leal, J., Valenzuela Suárez, L., Jayabalan, R., Huerta Oros, J., & Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites.

*CyTA - Journal of Food*, *16*(1), 390–399. https://doi.org/10.1080/19476337.2017.1410499

Matsuura, H., Hokura, A., Katsuki, F., Itoh, A., & Haraguchi, H. (2001). Multielement determination and speciation of major-to-trace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography. *Analytical Sciences*, *17*(3), 391–398. https://doi.org/10.2116/analsci.17.391

Mattes, R. D., & DiMeglio, D. (2001). Ethanol perception and ingestion. *Physiology & Behavior*, *72*(1–2), 217–229.

May, A., Narayanan, S., Alcock, J., Varsani, A., Maley, C., & Aktipis, A. (2019). Kombucha : A novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. *PeerJ*, *7*, e7565. https://doi.org/10.7717/peerj.7565

McEwan, J. A., & Colwill, J. S. (1996). The sensory assessment of the thirst-quenching characteristics of drinks. *Food Quality and Preference*, *7*(2), 101–111.

Monaco, E. (2019). 7 Easy ways to know if your kombucha is authentic (and why it matters). Organic Authority website: Retrieved from https://www.organicauthority.com/energetic-health/7-easy-ways-to-know-if-your-kombucha-is-authentic?fbclid=IwAR1H-sowA6ais Y9JonL62lvrp9Pv34eLppzj1hmwvVJkvIb0ciknwm3BRiM

Mordor Intelligence. (2019). Global kombucha market—growth, trends and forecast (2019—2024). Mordor Intelligence website: Retrieved from https://www.mordorintelligence.com/industry-reports/kombucha-market

Morrissey, W. F., Davenport, B., Querol, A., & Dobson, A. D. W. (2004). The role of indigenous yeasts in traditional Irish cider fermentations. *Journal of Applied Microbiology*, *97*(3), 647–655. https://doi.org/10.1111/j.1365-2672.2004.02354.x

Mossion, A. (2007). *Étude de la composition minérale et organique des liqueurs de thé et de leurs caractéristiques organoleptiques : Influence des paramètres physico-chimiques de l’eau.* (Doctoral Thesis). The Institut National Polytechnique de Toulouse, Toulouse, France, 213.

Mossion, A., Potin-Gautier, M., Delerue, S., Le Hécho, I., & Behra, P. (2008). Effect of water composition on aluminium, calcium and organic carbon extraction in tea infusions. *Food Chemistry*, *106*(4), 1467–1475. https://doi.org/10.1016/j.foodchem.2007.05.098

Murugesan, G. S. (2009). Hepatoprotective and curative properties of kombucha tea against carbon tetrachloride-induced toxicity. *Journal of Microbiology and Biotechnology*, *19*(4), 397–402. https://doi.org/10.4014/jmb.0806.374

Nahon, D. F., Roozen, J. P., & De Graaf, C. (1996). Sweetness flavour interactions in soft drinks. *Food Chemistry*, *56*(3), 283–289. https://doi.org/10.1016/0308-8146(96)00025-8

Neffe-Skocińska, K., Sionek, B., Ścibisz, I., & Kołożyn-Krajewska, D. (2017). Acid contents and the effect of fermentation condition of Kombucha tea beverages on physicochemical, microbiological and sensory properties. *CyTA - Journal of Food*, *15*(4), 601–607. https://doi.org/10.1080/19476337.2017.1321588

Nguyen, Nguyen K., Dong, N. T., Le, P. H., & Nguyen, H. T. (2014). Evaluation of the glucuronic acid production and other biological activities of fermented sweeten-black tea by kombucha layer and the co-culture with different *Lactobacillus* sp. Strains. *Ijmer*, *4*, 12–17.

Nguyen, N. K., Nguyen, P. B., Nguyen, H. T., & Le, P. H. (2015). Screening the optimal ratio of symbiosis between isolated yeast and acetic acid bacteria strain from traditional kombucha for high-level production of glucuronic acid. *LWT - Food Science and Technology*, *64*(2), 1149–1155. https://doi.org/10.1016/j.lwt.2015.07.018

Odake, S. (2001). Sweetness intensity in low-carbonated beverages. *Biomolecular Engineering*, *17*(4–5), 151–156. https://doi.org/10.1016/S1389-0344(01)00074-0

Ødegård, K. E., & Lund, W. (1997). Multi-element speciation of tea infusion using cation-exchange separation and size-exclusion chromatography in combination with inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, *12*(4), 403–408. https://doi.org/10.1039/A606153B

Alcoholic content, 27 C.F.R. § 7.26. (1993). Code of Federal Regulations,Title 27 : Alcohol, Tobacco and Firearms., T.D. ATF—339, 58 FR 21232, April 19, 1993.

Official Journal of the European Union. (2011). *Regulation (Eu) No 1169/2011 Of The European Parliament And Of The Council*

Paulus, K., & Reisch, A. M. (1980). The influence of temperature on the threshold values of primary tastes. *Chemical Senses*, *5*(1), 11–21. https://doi.org/10.1093/chemse/5.1.11

Peleg, H., Gacon, K., Schlich, P., & Noble, A. C. (1999). Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture*, *79*(8), 1123–1128.

Petrović, S. E., Suturović, Z. J., Lončar, E. S., & Malbaša, R. V. (1999). Potentiometric stripping analysis of certain metal ions in tea fungus beverage. *Nahrung/Food*, *43*(5), 345–346. https://doi.org/10.1002/(SICI)1521-3803(19991001)43:5<345::AID-FOOD345>3.0.CO;2-H

Petrucci, R. H., Herring, F. G., Madura, J. D., & Bissonnette, C. (2011). *General chemistry : Principles and modern applications* Toronto, Canada: Pearson.

Phan, T. G., Estell, J., Duggin, G., Beer, I., Smith, D., & Ferson, M. J. (1998). Lead poisoning from drinking Kombucha tea brewed in a ceramic pot. *Medical Journal of Australia*, *169*(11–12), 644–646.

Price, W. E., & Spitzer, J. C. (1994). The kinetics of extraction of individual flavanols and caffeine from a Japanese green tea (*Sen Cha Uji Tsuyu*) as a function of temperature. *Food Chemistry*, *50*(1), 19–23. https://doi.org/10.1016/0308-8146(94)90086-8

Price, W., & Spitzer, J. (1993). The temperature dependence of the rate of extraction of soluble constituents of black tea. *Food Chemistry*, *46*(2), 133–136. https://doi.org/10.1016/0308-8146(93)90025-B

Ramachandran, S., Fontanille, P., Pandey, A., & Larroche, C. (2006). Gluconic acid : Properties, applications and microbial production. *Food Technology and Biotechnology*, *44*(2), 185–195.

Redondo, N., Gomez-Martinez, S., & Marcos, A. (2014). Sensory attributes of soft drinks and their influence on consumers’ preferences. *Food & Function*, *5*, 1686–1694.

Reiss, J. (1994). Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, *198*(3), 258–261.

Reva, O. N., Zaets, I. E., Ovcharenko, L. P., Kukharenko, O. E., Shpylova, S. P., Podolich, O. V., … Kozyrovska, N. O. (2015). Metabarcoding of the kombucha microbial community grown in different microenvironments. *AMB Express*, *5*(1). https://doi.org/10.1186/s13568-015-0124-5

Robinson, J. M., & Owuor, P. O. (2013). Tea aroma. In K. C. Willson & M. N. Clifford. (Eds.), *Tea : Cultivation to consumption* ( pp. 603–647). Dordrecht: Springer.

Rosend, J., Kuldjärv, R., Rosenvald, S., & Paalme, T. (2019). The effects of apple variety, ripening stage, and yeast strain on the volatile composition of apple cider. *Heliyon*, *5*(6), e01953. https://doi.org/10.1016/j.heliyon.2019.e01953

Rubico, S. M., & McDaniel, M. R. (1992). Sensory evaluation of acids by free-choice profiling. *Chemical Senses*, *17*(3), 273–289. https://doi.org/10.1093/chemse/17.3.273

Shachman, M. (2005). *The soft drinks companion–a technical handbook for the beverage industry* Florida: CRC Press.

Shahbazi, H., Hashemi Gahruie, H., Golmakani, M.-T., Eskandari, M. H., & Movahedi, M. (2018). Effect of medicinal plant type and concentration on physicochemical, antioxidant, antimicrobial, and sensorial properties of kombucha. *Food Science & Nutrition*, *6*(8), 2568–2577. https://doi.org/10.1002/fsn3.873

Siebert, K. J. (1999). Modeling the flavor thresholds of organic acids in beer as a function of their molecular properties. *Food Quality and Preference*, *10*(2), 129–137.

Sieuwerts, S., Bron, P. A., & Smid, E. J. (2018). Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT*, *90*, 201–206. https://doi.org/10.1016/j.lwt.2017.12.022

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., & Teuber, M. (1995). Microbiology and fermentation balance in a kombucha beverage obtained from a tea fungus fermentation. *Systematic and Applied Microbiology*, *18*(4), 590–594.

Spiro, M., Jaganyi, D., & Broom, M. C. (1992). Kinetics and equilibria of tea infusion : Part 9. The rates and temperature coefficients of caffeine extraction from green Chun Mee and black Assam Bukial teas. *Food Chemistry*, *45*(5), 333–335. https://doi.org/10.1016/0308-8146(92)90033-X

Spiro, M., & Price, W. E. (1987). Kinetics and equilibria of tea infusion : Part 7—The effects of salts and of pH on the rate of extraction of caffeine from Kapchorua Pekoe Fannings. *Food Chemistry*, *25*(1), 49–59. https://doi.org/10.1016/0308-8146(87)90053-7

Spiro, M., Price, W. E., Miller, W. M., & Arami, M. (1987). Kinetics and equilibria of tea infusion : Part 8—the effects of salts and of pH on the rate of extraction of theaflavins from black tea leaf. *Food Chemistry*, *25*(2), 117–126. https://doi.org/10.1016/0308-8146(87)90060-4

Sreeramulu, G., Zhu, Y., & Knol, W. (2000). Kombucha fermentation and its antimicrobial activity. *Journal of Agricultural and Food Chemistry*, *48*(6), 2589–2594. https://doi.org/10.1021/jf991333m

Steinkraus, K. H., Shapiro, K. B., Hotchkiss, J. H., & Mortlock, R. P. (1996). Investigations into the antibiotic activity of tea fungus/kombucha beverage. *Engineering in Life Sciences*, *16*(2–3), 199–205.

Stillman, J. A. (1993). Color influences flavor identification in fruit-flavored beverages. *Journal of Food Science*, *58*(4), 810–812. https://doi.org/10.1111/j.1365-2621.1993.tb09364.x

Stone, H., & Oliver, S. M. (1969). Measurement of the relative sweetness of selected sweeteners and sweetener mixtures. *Journal of Food Science*, *34*(2), 215–222. https://doi.org/10.1111/j.1365-2621.1969.tb00922.x

Sung , H., Kole, A., Jones, H. D., Christensen, R., & Gladstein, J. (2009). A case of kombucha tea toxicity. *Journal of Intensive Care Medicine*, *24*(3), 205–207. https://doi.org/10.1177/0885066609332963

Talebi, M., Frink, L. A., Patil, R. A., & Armstrong, D. W. (2017). Examination of the varied and changing ethanol content of commercial kombucha products. *Food Analytical Methods*, *10*(12), 4062–4067. https://doi.org/10.1007/s12161-017-0980-5

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of Kombucha fermentation. *International Journal of Food Microbiology*, *95*(2), 119–126. https://doi.org/10.1016/j.ijfoodmicro.2003.12.020

Teranishi, R., Wick, E. L., & Hornstein, I. (Eds.). (1999). Progress of tea aroma chemistry. *Flavor chemistry: Thirty years of progress*, (pp. 135–145). New York, NY: Springer Science and Media.

Troitino, C. (2017). Kombucha 101 : Demystifying The Past, Present And Future Of The Fermented Tea Drink. Forbes website: Retrieved from https://www.forbes.com/sites/christinatroitino/2017/02/01/kombucha-101-demystifying-the-past-present-and-future-of-the-fermented-tea-drink/#2aa040b94ae2

Tse, H.-C. (1985). *United States Patent, Patent* No. *4,552,776*. Fairfield Ohio.

Valles, B., Bedrinana, R., Tascon, N., Simon, A., & Madrera, R. (2007). Yeast species associated with the spontaneous fermentation of cider. *Food Microbiology*, *24*(1), 25–31. https://doi.org/10.1016/j.fm.2006.04.001

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J.-P., & Taillandier, P. (2018). Understanding kombucha tea fermentation: A review. *Journal of Food Science*, *83*(3), 580–588. https://doi.org/10.1111/1750-3841.14068

Wang, C., Mas, A., & Esteve-Zarzoso, B. (2016). The Interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. *Frontiers in Microbiology*, *7*(502). https://doi.org/10.3389/fmicb.2016.00502

Wang, K., Gan, X., Tang, X., Wang, S., & Tan, H. (2010). Determination of d-saccharic acid-1,4-lactone from brewed kombucha broth by high-performance capillary electrophoresis. *Journal of Chromatography B*, *878*(3–4), 371–374. https://doi.org/10.1016/j.jchromb.2009.12.003

Wang, Y., Ji, B., Wu, W., Wang, R., Yang, Z., Zhang, D., & Tian, W. (2014). Hepatoprotective effects of kombucha tea : Identification of functional strains and quantification of functional components: Hepatoprotective effects of kombucha tea. *Journal of the Science of Food and Agriculture*, *94*(2), 265–272. https://doi.org/10.1002/jsfa.6245

Watson, E. (2019). What is an 'authentic' kombucha ? KBI gears up to publish definition. Food Navigator USA website: Retrieved from https://www.foodnavigator-usa.com/Article/2019/04/22/What-is-an-authentic-kombucha-KBI-gears-up-to-publish-definition?utm_source=newsletter_daily&utm_medium=email&utm_campaign=04-Jun-2019&c=QC7zMjzCaUsFlcwWw12hlr5WYPyT8dFn&p2=

Wei, J., Wang, S., Zhang, Y., Yuan, Y., & Yue, T. (2019). Characterization and screening of non-*Saccharomyces yeasts* used to produce fragrant cider. *LWT*, *107*, 191–198. https://doi.org/10.1016/j.lwt.2019.03.028

Willems, J. L., & Low, N. H. (2012). Major carbohydrate, polyol, and oligosaccharide profiles of agave syrup. application of this data to authenticity analysis. *Journal of Agricultural and Food Chemistry*, *60*(35), 8745–8754. https://doi.org/10.1021/jf3027342

Williams, A. A., & Rosser, P. R. (1981). Aroma enhancing effects of ethanol. *Chemical Senses*, *6*(2), 149–153. https://doi.org/10.1093/chemse/6.2.149

Wilt, P. M. (1986). Nucleation rates and bubble stability in water-carbon dioxide solutions. *Journal of Colloid and Interface Science*, *112*(2), 530–538. https://doi.org/10.1016/0021-9797(86)90122-0

Yamada, Y., Yukphan, P., Lan Vu, H. T., Muramatsu, Y., Ochaikul, D., Tanasupawat, S., & Nakagawa, Y. (2012). Description of *Komagataeibacter* gen. nov., with proposals of new combinations (*Acetobacteraceae*). *The Journal of General and Applied Microbiology*, *58*(5), 397–404. https://doi.org/10.2323/jgam.58.397

Yau, N. J. N., & McDaniel, M. R. (1992). Carbonation interactions with sweetness and sourness. *Journal of Food Science*, *57*(6), 1412–1416. https://doi.org/10.1111/j.1365-2621.1992.tb06871.x

Zara, S., Bakalinsky, A. T., Zara, G., Pirino, G., Demontis, M. A., & Budroni, M. (2005). FLO11-based model for air-liquid interfacial biofilm formation by *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, *71*(6), 2934–2939. https://doi.org/10.1128/AEM.71.6.2934-2939.2005

Zellner, D. A., & Durlach, P. (2002). What is refreshing ? An investigation of the color and other sensory attributes of refreshing foods and beverages. *Appetite*, *39*, 185–186.

Zhang, W., Wang, X., Qi, X., Ren, L., & Qiang, T. (2018). Isolation and identification of a bacterial cellulose synthesizing strain from kombucha in different conditions : *Gluconacetobacter xylinus* ZHCJ618. *Food Science and Biotechnology*, *27*(3), 705–713. https://doi.org/10.1007/s10068-018-0303-7

Zhou, Y., Zeng, L., Gui, J., Liao, Y., Li, J., Tang, J., … Yang, Z. (2017). Functional characterizations of *β*-glucosidases involved in aroma compound formation in tea (*Camellia sinensis*). *Food Research International*, *96*, 206–214. https://doi.org/10.1016/j.foodres.2017.03.049

Zhu, Q. Y., Zhang, A., Tsang, D., Huang, Y., & Chen, Z.-Y. (1997). Stability of green tea catechins. *Journal of Agricultural and Food Chemistry*, *45*(12), 4624–4628. https://doi.org/10.1021/jf9706080

**How to cite this article:** Tran T., Grandvalet C, Verdier F, Martin A, Alexandre H, Tourdot-Maréchal R. Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha. *Compr Rev Food Sci Food Saf*. 2020;19:2050–2070. https://doi.org/10.1111/1541-4337.12574