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Kombucha technology: production and legal aspects

Tecnologia do Kombuchá: produção e aspectos legais

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ABSTRACT

Kombucha is a fermented drink with growing interest among the population due to its health benefits. The technological process involved in the production of kombucha is complex and involves the symbiosis between yeast and bacteria. Our study aimed to review the literature and identify factors that influence the fermentation process through a narrative review of the literature. We observed that factors such as time, temperature, quantity and type of tea, geometry of the fermentation vessel and composition of bacteria and yeast influence the quality of the final drink, which can alter the drink's sensory characteristics and health benefits. We conclude that the existence and control of production parameters for the drink is essential, thus ensuring greater standardization and consumer safety.

Keywords: kombucha; scoby; fermented; green tea

RESUMO

A kombucha é uma bebida fermentada com crescente interesse da população devido aos seus benefícios à saúde. O processo tecnológico envolvido na produção da kombucha é complexo e envolve a simbiose entre leveduras e bactérias. Nosso estudo objetivou revisar a literatura e levantar fatores que influenciam no processo de fermentação através de uma revisão narrativa da literatura. Observamos que fatores como tempo, temperatura, quantidade e tipo de chá, geometria do vaso de fermentação e composição de bactérias e leveduras influenciam na qualidade da bebida final, podendo alterar as características sensoriais da bebida e os benefícios à saúde. Concluímos que é fundamental a existência e o controle de parâmetros de produção para a bebida garantindo assim maior padronização e segurança do consumidor.

Palavras-chave: kombucha; scoby; fermentados; chá verde

INTRODUCTION

Popularly, fermented foods are characterized as products obtained through the metabolic interaction of microorganisms and the conversion of components present in fermented bases. As part of this group, we have kombucha, a drink of oriental origin, originally fermented in *Cammelia sinensis* tea with a low final pH, which allows it to be preserved without the use of additives and with the nutritional benefits that tea presents (Jayabalan et al. 2008; Coton et al. 2017; Cardoso et al. 2020; Anantachoke et al. 2023).

Kombucha dates back to 220 BC in the Manchuria region in northeastern China. It has now regained prominence and has been widely disseminated in the global market for drinks and products with functional claims. This growth is due not only to the benefits associated with health, but also due to the associated sensory characteristics, such as carbonation and the countless possibilities for diversifying flavors. This ancient way of preparing and preserving drinks provides a product of excellent nutritional quality, even if we consider that the content of polyphenols and flavonoids tends to increase as the days of fermentation and metabolic interactions go by (Duta, Paul, 2019). Thus, it appears that fermentation allows for greater antioxidant activity when compared to unfermented tea, which is directly associated with the content of polyphenols and organic acids found in the final product (Jayabalan et al. 2008; Jayabalan et al. 2014; Coton et al. 2017).

The objective of this review was to survey the process of obtaining the drink, the characteristics of *Scoby*, creating theoretical interactions regarding the chemical characteristics and microbiological composition of the drink, the possible benefits and contraindications related to its consumption. Furthermore, we sought to describe some regulations imposed for its production and commercialization and finally, present the control factors that interfere in the fermentation process.

METHODOLOGY

The study is a narrative review of the literature, which in general, aims to elucidate the current state of knowledge on a specific subject through a synthesis, with the aim of identifying unexplored areas of knowledge, facilitating new investigations and laying the foundations for future research on a given topic. The non-systematic approach adopted in the narrative review model is simplified and seeks to provide a timely update on a given topic through broader and more extensive research, without adhering to a specific methodology for carrying out its various stages (Casarin et al. 2020). However, seeking to rely on a standardized line for data collection, even if not directly, using a collection alternative, based on the metadata proposed in the implementation of the *methodology Preferred Reporting Items for Systematic Reviews and Meta- Analyzes (PRISMA)*[1] in an adapted way, following the model proposed by Cardoso et al. (2022), which allowed the content covered to be revealed in a critical and concise manner, with the central objective of achieving prospection and elucidation of the topic in a systematic way, even if indirectly, making it possible to map current scientific and technological development, capable of impacting significantly, positively the industry, the economy and society.

[1]The PRISMA methodology is a set of guidelines for systematic reviews and meta-analyses, aiming to improve their quality and transparency. She provides guidance from the preparation of the protocol to the writing of the final report, promoting consistency and methodological rigor. PRISMA assists in the selection of studies, collection and synthesis of data, facilitating critical evaluation and replication of results.

OVERVIEW

Kombucha is a drink resulting from the biometabolic activity of a symbiotic culture of bacteria and yeast, which remains accommodated in a cellulose matrix, popularly known as *"Scoby – Symbiotic Culture of Bacteria and Yeast"*. In the beverage production process, the biochemical phenomenon of fermentation occurs, where transformations are generated in the tea as a result of the activity of microorganisms, leading to changes in phenolic compounds and the generation of organic acids, which include acetic acid, lactic acid and glucuronic acid (Vitas et al. 2013; Villarreal-Soto et al. 2019; Jakubczyk et al. 2020).

Among the claims attributed to the consumption of kombucha, the antimicrobial, antioxidant, and anticarcinogenic benefits have assumed a prominent role among the therapeutic properties and aroused consumer interest, enhancing its marketing profile (Chakravority et al. 2019; Cardoso et al. 2020; Anantachoke et al. 2023). The global kombucha production and commercialization market has demonstrated numerous advantages for significant growth and its dissemination on an industrial scale, highlighting it as the fastest growing product in the beverage sector with functional properties (Dutta and Paul, 2019).

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With the focus on the development of new drinks and promising products for the food market, accompanied by this trend of demand for more functional products, a marketing race was launched to offer packaged products for immediate consumption based on kombucha, requiring emerging form the implementation of specific legislation that establishes the Identity and Quality Standard (PIQ) of the product, as well as what information should be covered and contained in the means of dissemination and labeling of the product, making it safe for human consumption.

KOMBUCHA: PRODUCTION TECHNOLOGY

In the kombucha manufacturing process, teas derived from Camellia sinensis are the most used as a base, whether combined or isolated (Coelho et al. 2020; Cardoso et al. 2020). To start the fermentation process, in addition to the chosen base, a fraction of sucrose (5 to 12% (m/v)) is added, which will serve as an energy substrate for the metabolism of the microorganisms involved in the process, which may vary according to the standardization adopted in different processing conditions, acting in protocooperation (Dutta and Paul, 2019). At the beginning of fermentation, the disaccharide sucrose is hydrolyzed into smaller molecules of glucose and fructose, which will soon be bioconverted to ethanol and CO^2. Ethanol, in the spectrum of fermentation dynamics, is converted to acetic acid, with notable production of gluconic and glucuronic acids throughout the process.

The entire mechanism mentioned occurs spontaneously, which according to Coton et al. (2017) can be considered difficult to control, as there are different reactions, varying according to the microbiota involved and the production conditions, which promotes different results in the final drink. The fermentation process can vary from 3 to 60 days. With the union of the base raw material for the beverage production process, fermentation begins, where on average around 7 to 10 days are needed for the product to begin to acquire specific characteristics. This beginning is called first fermentation and, as it occurs, a new scoby film is formed on the surface of the solution. This cellulose biofilm can reach a thickness of between 8 and 12 mm (Chandrakala, Lobo and Dias, 2019). The process takes place at a variable temperature, knowing that between 25 – 30 ºC is ideal. The fermentation process occurs even at lower temperatures, but the fermentation speed is slower, taking longer for the drink to acquire the same sensory characteristics (Leal et al. 2018; May et al. 2019).

During this period, invertase enzymes, produced by some species of yeast, act to cleavage sucrose molecules, releasing glucose and fructose into the solution, increasing the supply of substrates for metabolism. Glucose molecules are used by bacteria and yeast in energy metabolism, transforming into ethanol and carbon dioxide (May et al. 2019), this carbon dioxide will undergo a reaction with the water in the solution medium, forming carbonic acid (Primiani et al. 2018). Furthermore, species of microorganism's act in the oxidation of ethanol, producing acetic acid.

It appears that in addition to acetic acid, several other organic acids are produced throughout the fermentation process, which contributes to the hydrogen potential (pH) of the drink, which initially hovers around 5, decreasing even further (Amarasingkhe, 2018; May et al. 2019). This low pH characteristic of the drink is responsible for conservation without the need for additives, being seen as a very important point of the drink in terms of becoming healthier. Organic acids, D-saccharic acid-1,4-lactone (DSL), and tea polyphenols are the main metabolite components of kombucha (Jayabalan et al. 2014).

On an artisanal and industrial scale, scoby is added to the tea solution with sucrose in a container that can be made of glass or stainless steel. This container must have a wide neck, with an extended exposure area, allowing easy access and surface exposure, sufficient for the exchange of gases with the environment. Emphasizing that this bottleneck must be covered with a protection that is permeable to these atmospheric gases, but that prevents the entry of insects (Jayabalan et al. 2014).

The new fraction of the cellulose film formed on the surface of the container is removed, and the liquid fraction is filtered and the drink is finished for consumption, and can be refrigerated or continued for a second fermentation (Watawana et al. 2015). In this second fermentation, this is where 10 - 50% of fruit juice, spices or other ingredients are generally added to the previously fermented kombucha (7-15 days). each trademark. This second fermentation aims to carbonate the kombucha, which must be kept in a closed container (anaerobic fermentation) and stored at room temperature until the desired degree of carbonation that meets legal standards (Santos et al. 2016). The result of this process is a drink that has a slightly carbonated characteristic, in addition to being very refreshing, composed of several elements beneficial to health (Watawana et al. 2015; Leal et al. 2017; May et al. 2019).

DELINEATION OF CONTROL PARAMETERS DURING FERMENTATION DYNAMICS

Most fermentation processes are aerobiosis and therefore require the supply of oxygen for metabolism to occur. Considering the stoichiometry of respiration, 192 g of oxygen are required for the complete oxidation of 180 g of glucose. However, both components must be in solution before they are available to a microorganism, and oxygen is approximately 6000 times less soluble in water than glucose, so it is not possible to provide a microbial culture with the necessary amount of oxygen to complete the oxidation of glucose or any other carbon source (Villareal-Soto et al. 2019).

At the beginning of the process, significant amounts of ethanol and monosaccharides necessary for lactic acid bacteria (AAB) are supplied by Kombucha yeasts. The oxidation of ethanol to acetic acid requires one mole of oxygen (32 g) to completely oxidize 1 mole of ethanol (46 g), therefore the activity of AAB as aerobic organisms depends on the transfer of oxygen from the air for fermentation. For this reason, a microbial culture must receive oxygen during growth at a rate sufficient to satisfy the organism's demand (Stanbury et al. 2016).

In static cultures, substrates must be entirely transported by diffusion and oxygen availability can become the limiting factor for cellular metabolism, which can have a negative effect on cellulose production and quality. The kinetic factor that expresses the relationship between dissolved oxygen and the surface/volume of the medium is the specific interfacial area, which is directly related to other factors, such as the cross section of the reactor and the mass transfer coefficient (Cvetković et al. 2008). This means that the rate of batch fermentation of Kombucha without stirring and without introducing gas depends on the specific interfacial area (which further promotes the idea of the fermentation environment being favorable in relation to the surface area).

Cvetković et al. (2008) developed a mathematical model to scale the fermentation of Kombucha tea based on several specific interface areas. Model verification was carried out in large volume reactors (90 L) and very small vessels of 0.33 L. The model standardizes the optimal conditions as: 70 g/L of initial substrate (sucrose), interfacial area of 0.0231 at 0.0642 cm^{-1} and 14 days of fermentation. They concluded that regardless of the size or volume of the container, if the value of the interfacial area is constant, they can guarantee the production of Kombucha tea with similar properties. In the specific case of batch fermentation of Kombucha tea, several biological factors must be taken into consideration, especially in the absence of agitation, where microbial disintegration can occur between the aerobic acetic bacteria that will tend to occupy the surface layer and the yeast that can precipitate at the bottom of the container (Lončar et al. 2006), which can lead to negative effects on the fermentation process.

Microbial cellulose has already been well studied by some authors over the last few decades (Czaja et al. 2006; Campano et al. 2016), and the available information that defines the optimal reactor conditions for its development, such as surface/volume or surface/height are limited. To investigate the influence of volume on processing, Lončar et al. (2006) worked with different conditions and found that the best geometric conditions for intensifying fermentation were obtained with a reactor with a volume of 4L and a diameter of 17 cm. Goh et al. (2012) investigated the relationship between the yield, the properties of the biofilm produced from Kombucha fermentation and the surface area and found that biofilm production increased with an intensification of the surface area and decreased with a wider depth. This can be explained because the metabolic process is completely aerobic and is constantly generating carbon dioxide, which can be trapped in the film and accumulate in greater quantities, especially in deeper environments.

However Caicedo, Da França & Lopez (2001) found that although the surface area is decisive, the height is still important, as a minimum height is necessary for the development of the film, taking into account the production of several layers of cellulose throughout fermentation, which will occupy part of the initial volume. Permeating the fermentation pattern, it is noted that there are many variables that interfere in the fermentation process and, consequently, in the sensorial characteristics of the drink. In this way, different final products are offered to the consumer and the responses in relation to the health benefits and also the acceptance of the drink, making it necessary for standards and regulations to be established to guarantee the quality of the product that reaches the consumer (Dutta and Paul, 2019).

Throughout this process, there are several factors involved in the excellence of the fermentation, as well as the quality of the product obtained at the end, which permeate the quality of the extract used as a liquid base for the culture, as well as the microorganisms, which make up the structure of the scoby. The type of substrate selected, the time and temperature released and the pH of the medium, are relevant factors that directly imply how this mechanism presented so far will develop (Villareal-Soto et al. 2018). Frame 1 presents different time and temperature conditions adopted in the fermentation of kombucha in several studies cited in the literature, which used different base and control parameters.

Frame 1 - Time and temperature conditions adopted in the fermentation of kombucha in several studies in the scientific literature

Fermentation time	Temperature used (°C)	Reference
21 days	24 ºC	Jayabalan et al. (2010)
10 days	28 ºC	Malbaša et al. (2011)
8 days	30 ºC	Goh et al. (2012)
10 days	23 ºC	Marsh et al. (2014)
10 days	30 ºC	Lončar et al. (2014)
8 days	Room temperature	Nguyen et al. (2015)
21 days	28 ºC	Chakravortya et al. (2016)
12 days	30 ºC	Ayed; Abid; Hamdi, (2017)
21 days	20ºC (CHV) e 30º (CHP)	Filippis *et al.* (2018)

Caption: CHV = green tea; CHP = black tea.
Source: Authors, 2024.

May et al. (2019) states that, when carbon levels are very low, new substrates in addition to sucrose can begin to be used by bacteria and yeast, envisioning alternative routes for obtaining carbon molecules, such as ethanol. However, it should be noted that such alternative mechanisms also impact the metabolites produced throughout fermentation. It is known that sucrose is the carbon source most used in this process, however, an important fraction of this substrate is not used during fermentative metabolism (Jayabalan et al. 2014) and when adding other types of sugar, such as lactose or fructose, can have impacts on fermentation and the final product.

In the study by Malbaša, Lončar and Djurić (2008), in which the elaboration of variations of kombuchas fermented in solutions prepared by the infusion of black tea and sucrose and black tea and sugarcane molasses, it was observed that in the drink prepared with the addition of sucrose as the main carbon source, a greater production of acetic acid was identified, which was proportionally associated with a reduction in the pH values of the medium. In the sample that used sugarcane molasses as an energy substrate to obtain carbon, a higher concentration of lactic acid was noted.

Traditionally, pH tends to decrease during the fermentation process, this parameter being related to safety against the growth of pathogenic species of microorganisms, with the inhibition range plotted, normally at pHs close to 4.2 (Hur et al. 2014; May et al. 2019). Jayabalan et al. (2014) point out that the pH range of kombucha tends to decrease mainly in the initial days of fermentation, tending to stabilize due to the buffering effect exerted by the acids in the solution. Malbasa et al. (2011) explain that this phenomenon of "plateau" of equilibrium at a given moment of fermentation, due to buffering, is due to the fact that the synthesis of weak organic acids interacts with mineral components present in the solution, mainly those derived from tea.

In the broad commercial spectrum, the final beverage is composed of sugars (including sucrose, glucose and fructose), organic acids including acetic acid, gluconic acid, glucuronic acid, lactic acid, DSL, citric acid, oxalic acid and pyruvic acid (Jayabalan et al. 2007) B vitamins and vitamin C (Bauer-Petrovska and Petrushevska-Tozi, 2000), theophyllines, tea polyphenols, flavonoids, various amino acids and proteins, ethanol, biogenic amines, purine bases, hydrolytic enzymes, minerals (mainly copper (Cu), Iron (Fe), Zinc (Zn), Nickel (Ni) and Manganese (Mn)) and metabolites secreted by yeast and bacteria (Malbaša et al. 2011).

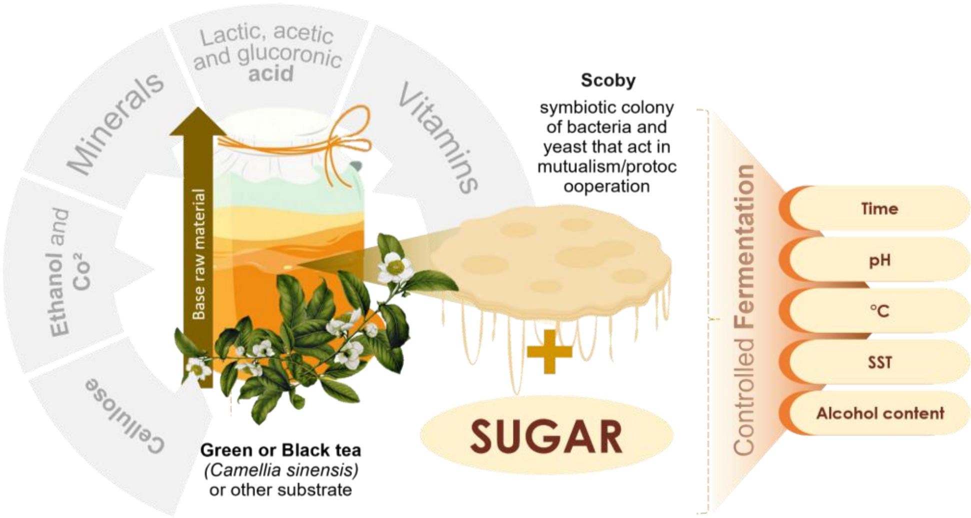
Tea itself contains the main polyphenolic catechins described in the literature, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). Black tea is considered a fully fermented form of tea as the production process creates a small particle size in the tea leaves and a larger surface area for enzymatic oxidation. During the fermentation of black tea, quinones react with catechins and produce new compounds, such as theaflavins and thearubigins. Furthermore, the catechins present in green tea are also partially converted into theaflavins (Haruthairat Kitwetcharoen et al. 2023).

The phenolic compounds in kombucha are bioconverted into smaller biological molecules, through the fermentation process that occurs in an acidic environment or through enzymatic activity, guided by the metabolism of bacteria and yeast. For example, the observed increase in overall catechin content in green and black tea kombucha subsequent to fermentation can be attributed to the biotransformation process in which *epigallocatechin-3-gallate* (EGCG) undergoes conversion to *epigallocatechin gallate* (ECG) and *epicatechin* (EC), by enzymes released by microbial communities in an acidic environment. The mechanism involved occurs by the hydrolysis of EGCG into smaller molecules and converted into *epigallocatechin* (EGC), EGCG and EC (Jayabalan et al. 2008). Other molecules such as theaflavins and thearubigin, which are derived from the polyphenol complex found in black tea and are associated with color changes in tea, are also found (Martínez-Leal et al. 2018). It is found that the lighter color of a fully fermented kombucha can be attributed to the conversion of theaflavins into theobromine (Jayabalan et al. 2007; Torre et al. 2021).

In the scientific field, it is possible to verify the use of other means of solution, used as alternative bases for the production of the drink, for example, wheatgrass juice

(Sun, Li, Chen, 2015); soy whey (Tu et al. 2019), matte tea (Santos et al. 2009), coffee (Watawana et al. 2015) and coconut water (Villareal-Soto et al. 2018). Figure 1 briefly presents the main actors involved in the obtaining process and the main components found in kombucha.

Figure 1 - Components involved in the obtaining process and the main components found in kombucha



pH: hydrogen potential; °C: Degrees Celsius; SST: Total soluble solids (°Brix); CO²: Carbon Dioxide.
Source: Authors, 2024.

DYNAMICS OF PROTOCOOPERATION OF THE MICROBIOLOGICAL ECOSYSTEM AND OBTAINING THE DRINK

The complexity of kombucha fermentation kinetics occurs mainly due to the number of microorganisms involved and their interactions (Kitwetcharoen et al. 2023). Most species excrete metabolic products that can stimulate or inhibit the specific growth rate of other species, establishing commensalistic or amensalistic interactions that require a clear understanding of this coexistence phenomenon. Some groups of bacteria such as lactic acid and acetic acid bacteria (LAB and AAB), as well as yeast species such as Saccharomyces cerevisiae, have well-established roles in this dynamic.

The complexity of the system encompasses countless species that are still unknown, both in relation to their genetics and bioactivity, as well as their interactions, and some obstacles prevent the complete understanding of these ecosystems, with this diversity and complexity of microbial communities being the first of them, mainly in the delimitation of such as, some microorganisms can act in parallel, while others act sequentially with dominant evolution during the fermentation process (Chakravorty et al. 2016). Specifically, when dealing with kombucha, the supernatant biofilm of microorganisms *(scoby)* is used as a key and essential element for the occurrence and maintenance of this process, having a unique and very complex microbiota, which can be divided into two fractions, namely, one recognized in biological material and the other, found in the drink itself (Chakravority et al. 2016). The main bacteria identified in scoby are the following strains: *Acetobacter xylinodes, komagataeibacter xylinus, Gluconacetobacter xylinus, Acetobacter pasteurianus and Acetobacter aceti* (Dutta and Paul, 2019).

Jayabalan et al. 2007 point out that a range of yeasts have also been identified as elements present in the composition of scoby, including *Saccharomyces, Saccharomicodes, Schizosaccharomyces, Brettanomyces/Dekkera, Candida, Torulospora, Koleckera, Pichia, Mycotorula and Mycoderma*, promoting the idea that in addition to bacteria acetic yeasts normally cited as elements present in the composition, there are many species of yeast, with traditionally recognized strains. What was encouraged by Chakravorty et al. (2016), who evaluated the microbiological and chemical profile of kombucha, using modern genetic sequencing tools and observed that the genus Candida was the predominant yeast in the two portions evaluated (biofilm and liquid) of the kombucha used for the study. In disagreement, Marsh et al. 2014, when analyzing a sample of kombucha from the perspective of microbiology, showed the dominance of Saccharomyces and Zygosaccharomyces in the analyzed samples, with the first yeast being found in very low proportions and the second not found in other studies, such as that of Chakravorty et al. (2016). In a comprehensive way, Table 1 presents some of the microorganisms found mapped in the drink and in the scooby according to the scientific literature mapped.

BRAZILIAN LEGISLATION: ADOPTION OF IDENTITY AND QUALITY STANDARDS (PIQ) AS AN ELEMENT OF SECURITY FOR THE INDUSTRY AND THE CONSUMER

The production of metabolites such as ethanol and acetic acid in kombucha acts preventively on the growth of microorganisms with pathogenic potential, inhibiting their growth by modifying the culture medium. However, during the beverage production process, having faithful control over the physical-chemical parameters of the product is essential. For the food industry to guarantee the production of a drink or food with quality and safety, it must meet the standards established by regulatory agencies in the national territory, such as the Ministry of Agriculture, Livestock and Supply (MAPA) and the National Health Surveillance Agency (ANVISA).

Following this trend in the population's consumption pattern, added to the great interest in expanding fermentation processes at an industrial level, MAPA, in June 2018, published a public consultation, over a period of 75 days, exposing the scope of Ordinance No. 64, of May 14, 2018, in which it provides for the project to implement the Normative Instruction (IN), which aims to define the Identity and Quality Standard (PIQ) specific to kombucha throughout the national territory.

The result of this public call was Normative Instruction nº41/2019 (Brasil, 2019), published in September 2019, in the Official Gazette of the Union. The publication of this normative instruction placed Brazil in the position of the first country in the world to have a specific standard regarding the characteristics of kombucha, establishing and demanding standards from manufacturers.

IN nº41/19 defines kombucha as “The fermented drink obtained through aerobic respiration and anaerobic fermentation of the must obtained by the infusion or extract of Camellia sinensis and sugars by symbiotic culture of microbiologically active bacteria and yeasts *(scoby)* (Brazil, 2019). The entire scope of standards established in this IN apply only to kombuchas obtained industrially, where technologically adequate processing is expected, meeting the established parameters.

Kombucha must be composed of drinking water, infusion or aqueous extract of *Camellia sinensis*, sugars, according to specific ANVISA legislation (Brazil, 2005), in addition to the symbiotic culture of bacteria and yeast *(Scoby)* suitable for alcoholic and acetic fermentation, as long as its safety to human health is guaranteed. The analytical parameters of kombucha, required by Brazilian legislation, are shown in Frame 2.

Table 1 - Main microorganisms found in kombucha and scoby according to scientific literature.

YEAST		BACTER
Arxula adeninivorans	*Meyerozyma caribbic*	*Acetobacter aceti*
Brettanomyces lambicus	*Meyerozyma guilliermondii*	*Acetobacter nitrogenifigens*
Brettanomyces claussenii	*Mycoderma sp.*	*Acetobacter okinawensis*
Brettanomyces custersii	*Mycotorula sp.*	*Acetobacter pasteurianus*
Brettanomyces/dekkera anômala	*Saccharomyces cerevisiae*	*Acetobacter peroxydans*
Brettanomyces/dekkera bruxellensis	*Saccharomyces ludwig Naumovozyma*	*Acetobacter sp.*
Cândida índia	*Pichia fermentans*	*Acetobacter syzygii*
Cândida sp.	*Pichia membranifaciens*	*Acetobacter tropicalis*
Candida kefyr	*Pichia mexicana*	*Bacterium gluconicum*
Candida krusei	*Pichia sp.*	*Bifidobacterium*
Candida stellata	*Saccharomyces uvarum*	*Collinsella*
Candida stellimalicola	*Saccharomycodes ludwigii*	*Enterobacter cancerogenus*
Candida tropicalis	*Saccharomycopsis fibuligera*	*Enterobacter cloacae*
Candida parapsilosis	*Schizosaccharomyces pombe*	*Enterobacter ludwigii.*
Debaryomyces hansenii	*Sporopachydermialactativor*	*Gluconacetobacter europaeus*
Dekkera anomala	*Starmeraamethionina*	*Gluconacetobacter intermedius*
Dekkera bruxellensis	*Starmeracaribae*	*Gluconacetobacter kombuchae*
Eremothecium ashbyii	*Torulaspora delbrueckii*	*Gluconacetobacter rhaeticus*
Eremothecium cymbalariae	*Torulopsis sp.*	*Gluconacetobacter sp.*
Halomonas sp.	*Zygosaccharomyces bailii*	*Gluconacetobacter xylinus*
Hanseniaspora uvarum	*Zygotorulaspora florentina*	*Gluconobacter entanii*
Hanseniaspora meyeri	*Zygowilliopsis californica*	*Gluconobacter oxydans*
Hanseniaspora valbyensis		*Gluconobacter saccharivorans*
Hanseniaspora vineae		*Gluconobacter sp.*
Herbaspirillum sp.		*Komagataeibacter hansenii*
Kazachstania telluris		*Komagataeibacter rhaeticuse*
Kazachstania exigua		*Komagataeibacter xylinus*
Kloeckera apiculata		*Lactobacillus fermentum*
Kluyveromyces marxianus		*Lactobacillus nagelii*
Lachancea thermotolerans		*Lactobacillus satsumensis*
Lachancea fermentati		*Lactococcus*
Lachancea kluyveri		*Oenococcus oeni*
Leucosporidiella		*Propoonibacterium*
Merimblaingelheimense		*Ruminococcaceae incertae sedis*
		Weissella

Source: Marsh et al. 2014; Reva et al. 2015; Chakravorty et al. 2016; Coton et al. 2017; Gaggia et al. 2018, 2019; Sinir, Tamer and Suna, 2019; Dutta & Paul, 2019.

Frame 2 - Control parameters included in IN nº 41 of September 17, 2019, which regulates the PIQ of kombucha.

Parameter	**Minimum**	**Maximum**
Hydrogenion potential (pH)	2,5	4,2
Alcohol content (% v/v) alcohol-free kombucha	-	0,5
Alcohol content (% v/v) kombucha with alcohol	0,6	8,0
Volatile acidity (mEq/L)	30	130
Pressure (atm at 20ºC) in kombucha added with CO2	1,1	3,9

Source: Brazil, 2019.

In addition to the required parameters, IN nº 41/19 contains other inspection and care rules, which aim to guarantee the safety of the product for human health. In finished kombucha, the presence of viable microorganisms may occur, due to the detachment of scoby fractions, and their addition to the packaged product after the fermentation process is strictly prohibited (Brasil, 2019). In order to overcome this impasse, the legislation authorizes the use of technological processes, such as pasteurization, filtration, centrifugation, among others.

Pasteurization occurs by raising and reducing the temperature of the product, leading to a reduction in the microbiological load after fermentation, with the aim of maintaining a degree of stability to the growth of microorganisms naturally present in the drink, which present a potential for toxicity to the individual. Furthermore, even if the presence of viable microorganisms is verified, the product cannot exploit this characteristic, pointing out supposed functional properties or health benefits, in labeling and/or advertising, resulting in legal administrative measures provided for within the scope of legislation (Brazil, 2019).

In the analysis guided by Alencar et al. (2020) in which they sought to evaluate the suitability of kombucha labels sold in Brazilian territory, it was observed that of the different brands analyzed, the vast majority had some characteristic that did not comply with specific legislation (IN nº 41/2019). Of the products analyzed, the following names were identified in the label structure: “fermented drink”, “probiotic drink”, “live drink”, “Kombucha tea”, “Kombucha culture”, “symbiotic colony” and “live organisms”. The authors highlight that this diversity in terms of drink names can be explained by the lack of information held by manufacturers, resulting from gaps in current legislation for characterizing this drink, which leads to highlighting the importance of legislation on food labeling (Alencar et al. 2020).

In addition to bringing a new name to the product, the regulations also align topics highlighting what can be optionally used as an additional ingredient in the beverage manufacturing process, as long as they are included on the label, such as: the infusion of plant species in water or its extracts, fruits, vegetables, spices, honey, molasses and other sugars of vegetable origin, in addition to other components provided for in ANVISA legislation (RDC nº 54 of November 12, 2012), such as fibers, salts minerals and carbon dioxide (CO^2) used in industrial carbonation (Brazil, 2012; 2019).

Adding any ingredient that is not permitted, according to current specific ANVISA legislation, may be considered an adulterant product. The body approved the use of new ingredients that can be used in the beverage manufacturing process, including natural colorings and flavoring compounds, in accordance with RDC nº. 2, of January 15, 2007 and nº. 5, of January 15, 2007 for non-alcoholic kombucha. Adding volatile acids, synthetic or from exogenous sources, which do not come exclusively from the fermentative process of inputs and bacterial metabolism, constitutes a breach in the scope of the legislation and is characterized as a crime of industrial responsibility, with MAPA being responsible for registering and monitoring the establishments and products, ensuring that they comply with current legislation (Brazil, 2007).

CONCLUSION

Kombucha presents itself as a fermented drink with growing popularity, driven by its sensorial characteristics and potential health benefits. The production process, although traditional, involves a complex interaction of bacteria and yeast, resulting in a unique microbiological ecosystem that requires rigorous control to guarantee the safety and quality of the final product.

Research into kombucha, encompassing several areas of knowledge, is fundamental to elucidate the fermentation mechanisms, optimize the production process, characterize the composition and bioactivity of the drink, and explore new bases and ingredients. Furthermore, studies on health benefits, including antioxidant, antimicrobial and anticarcinogenic properties, are crucial to substantiate functional claims and ensure safe consumption.

The development of the kombucha industry in Brazil depends on the synergy between research, technological innovation and regulatory rigor, driving the production of a safe, highquality drink with the potential to add value to the production chain and promote the health of the population.

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