

The Kombucha phenomenon: production aspects, health benefits, and food safety issues

O fenômeno da Kombucha: aspectos de produção, benefícios à saúde e aspectos de segurança alimentar

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ABSTRACT

The artisanal production of kombucha has caused the spread of manufacturing and worldwide consumption in the last ten years. The characteristics similar to carbonated beverages and their bioactive and functional properties have attracted the staggering of industrial production, however, the process control and food safety make the beverage production challenges. The objective is to carry out a bibliographic review of the functional aspects of beverage consumption, as well as the fermentation control parameters mentioned by some authors. The search for articles was carried out in the Scopus database using the Bibliometrix tool to select the terms used, to contextualize the subject about the evolution of consumption and artisanal production, benefits, and food security. It was found that fermentation conditions, the type of tea used, and the concentration of sucrose are determining factors in some bioactive properties and sensory characteristics of kombucha. The benefits of consumption are greater with its harm, the harm being rare and related to symptoms similar to food poisoning. This factor is decisive for further studies related to the drink's food security. Even with benefits, there is a need

for the dissemination of scientific information on the production of the drink, as it is clear that there is a relationship between microbiological growth on the surface of the fermented liquid, also called SCOBY, and the presence of acetic acid bacteria negative (AAB).

Keywords: Food Safety, Fermentation, Tea Beverages, Parameters.

RESUMO

A produção artesanal do kombucha tem causado a disseminação da fabricação e do consumo mundial nos últimos dez anos. As características da kombucha, semelhantes às bebidas carbonatadas, e suas propriedades bioativas e funcionais têm trazido a necessidade do escalonamento da produção industrial, porém, o controle do processo e a segurança alimentar tornam a produção destas bebidas um desafio. O objetivo é realizar uma revisão bibliográfica sobre os aspectos funcionais do consumo de bebidas, bem como os parâmetros de controle da fermentação citados por alguns autores. A busca de artigos foi realizada na base de dados Scopus por meio da ferramenta Bibliometrix para selecionar os termos utilizados, contextualizar o assunto sobre a evolução do consumo e da produção artesanal, benefícios e segurança alimentar. Verificou-se que as condições de fermentação, o tipo de chá utilizado e a concentração de sacarose são fatores determinantes em algumas propriedades bioativas e características sensoriais do kombucha. Os benefícios do consumo são maiores do que os relatos de prejuízos, sendo os danos raros e relacionados a sintomas semelhantes aos de intoxicação alimentar. Esse fator é decisivo para novos estudos relacionados à segurança alimentar da bebida. Mesmo com benefícios, há necessidade de divulgação de informações científicas sobre a produção da bebida, buscando demonstrar as relações entre o crescimento microbológico na superfície do líquido fermentado, também denominado SCOBY, e a presença de acético bactérias ácidas negativas (AAB).

Palavras-chave: Segurança Alimentar, Fermentação, Chá, Bebidas, Parâmetros.

1 INTRODUCTION

In recent times, the search for healthier foods has resulted in a change in the profile of consumers, who have started to assess the nutritional, sensory aspects, and purposes of the foods they consume. In this context, the search for functional foods and beverages responsible for promoting beneficial effects on human health stands out [1]. Kombucha is one of these drinks and its spread, both in terms of its artisanal production and its consumption, is significant.

Kombucha is a drink of Asian origin resulting from the fermentation of black tea or green tea to which is added a symbiotic association of bacteria capable of carrying out various biochemical reactions. It has a sweet taste, slight acidity, and is slightly carbonated [2]. Due to the bioactive and functional characteristics of the drink, several benefits have been reported on its consumption, especially the antioxidant effect, in addition to becoming a potential substitute for soft drinks and alcoholic beverages,

becoming a worldwide trend. During its production, different fermentative parameters must be controlled, such as the type of tea, the initial concentration of sugar and inoculum, and the fermentation time [3]. However, these factors are not yet standardized, mainly because it is a drink that can be handcrafted.

Kombucha is a complex mixture of microorganisms so it is essential to discuss the food safety of tea for direct consumption. Although innumerable reports show the therapeutic benefits of the drink, there is evidence related to the concern of its toxicity and health risks.

This article presents a bibliographic review that aims at the global contextualization of kombucha drinks. Emphasizing the functional aspects, parameters of fermentative control, and food security of the drink, as well as the benefits and possible harms of its consumption. For the selection of articles, the Scopus database was used, which was explored in the “Bibliometrix” tool.

2 METHODOLOGY

For the selection of articles relevant to the development of the work, the Scopus database was used. According to Pagani, Kovaleski and Resende [4], the use of databases such as Scopus allows research to be carried out more reliably and safely.

Initially, articles were searched on Scopus that addressed the term “kombucha” in the title, abstract, and keywords, without adding filters for the global context of the subject. The resulting research was explored using the “Bibliometrix” tool in RStudio® software version 7.6. In all, 445 documents were identified with the presence of the term and it was possible to observe that there was an increase in annual and worldwide scientific production in the last 20 years.

The “Bibliometrix” tool also made it possible to identify the main content-generating countries with the theme “kombucha”. Western countries like Iran and India are responsible for the largest number of documents due to the origin of the drink, as well as high consumption. According to Fig. 1, Brazil is ranked 7th in the production of scientific documents on the subject. Fig. 2 shows the word cloud most cited by the authors.

Figure 1 Scientific production on the kombucha theme by country

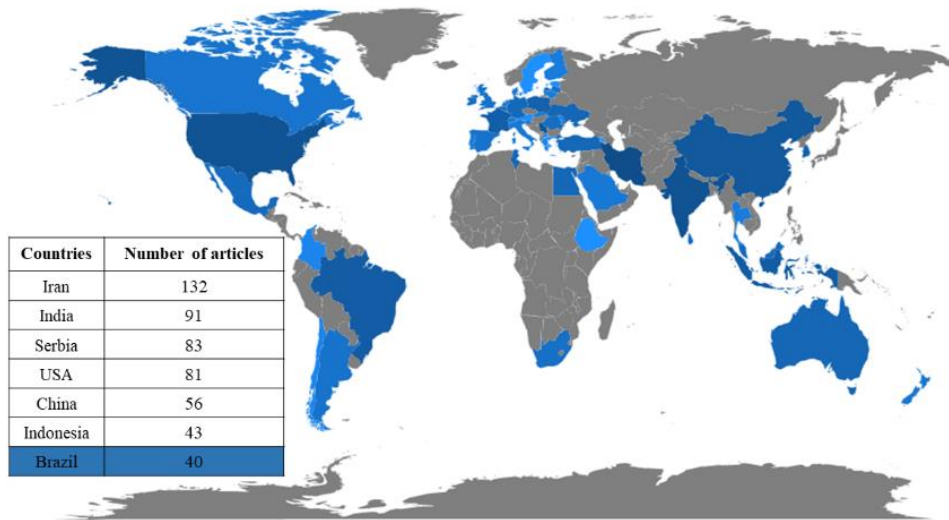
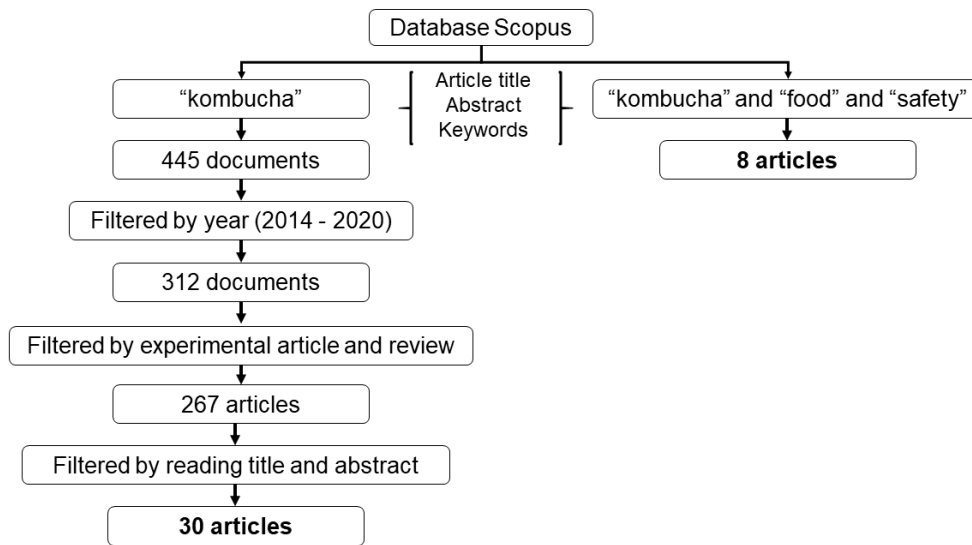


Figure 2 Word cloud filtered by author's keywords



From there, articles were selected for the development of the work, filtering the research for the last 6 years and reading the articles where the title and summary contained in the production process parameters and also which addressed the benefits of the drink to health. After this analysis, a second analysis was carried out with the terms "kombucha", "food" and "safety" in the title, abstract, and keywords, resulting in 8 documents. It was found that publications involving food security in the production of kombucha are a trend, as well as the concern with the microbial composition of the drink. The methodology for selecting the documents used in this review is shown in Fig. 3.

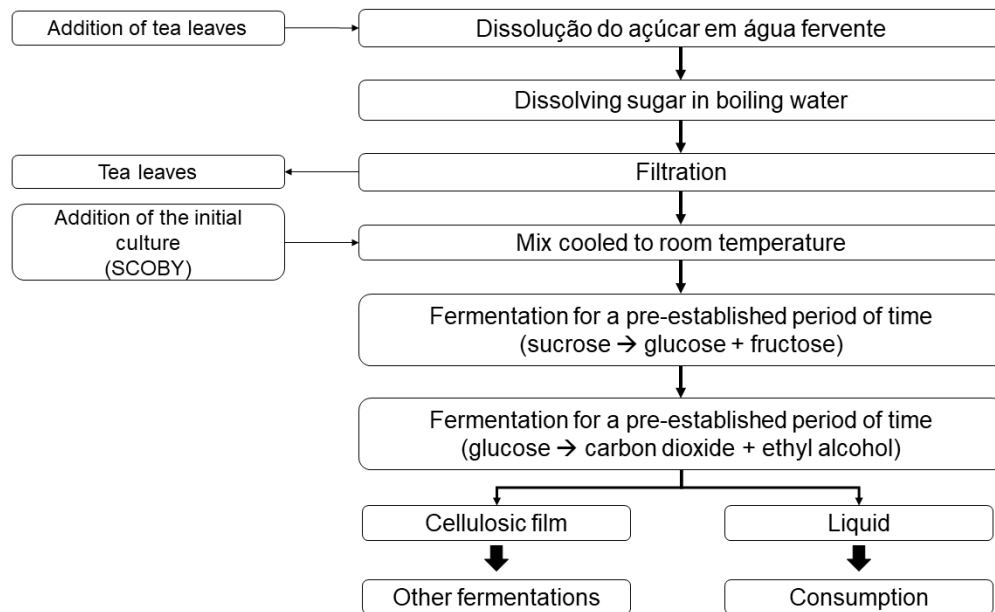
Figure 3 Flowchart of the methodology for selecting articles relevant to the development of the work



3 PRODUCTION PROCESS AND FERMENTATIVE PARAMETERS

Kombucha is produced by aerobic fermentation of both black and green tea, with black tea traditionally used. Sucrose is used as a carbon source, conventional sugar is normally used, and fermentation occurs by combining a symbiotic culture of bacteria and yeast known as SCOBY (symbiotic culture of bacteria and yeast). Yeast converts the available carbon source into ethanol, which is later converted to acetic acid and acetaldehyde by bacteria. The pH of the drink decreases due to the production of organic acids during fermentation [3,5], contributing to the refreshing sensory characteristics. Figure 4 shows a schematic of the steps in the process to obtain kombucha and it can be observed that during the fermentation process, sucrose is degraded through enzymes, produced by yeasts present in SCOBY, and converted into glucose and fructose. This first fermentation stage takes place in a semi-open environment for air exchange. Subsequently, in a closed environment, the second fermentative stage occurs, in which yeasts transform glucose into carbon dioxide and ethyl alcohol, the main sources for the production of acids responsible for the sensory characteristics of the product. Also, acetic bacteria use sucrose as a carbon source for the production of a cellulosic film, also called by some authors biofilm, as a secondary metabolite of fermentation, giving rise to a new SCOBY.

Figure 4 Kombucha production process steps



Kombucha is used as a probiotic drink, due to its function in facilitating the digestion and absorption of nutrients and being considered by some authors as a symbiotic food because it has strains with probiotic potential [6,7]. Symbiotic foods, according to Gibson and Roberfroid [8], are a mixture of prebiotics and probiotics that beneficially affect the host, improving the survival and implantation of live microbial food supplements in the gastrointestinal tract, selectively stimulating the growth of one or a limited number of health-promoting bacteria, thereby improving the health of the host. However, there are no studies on the effect of kombucha on gastrointestinal and microbiota health linked directly to humans [3].

The potential benefits of kombucha for human health are strongly linked to the existence of antioxidants, such as polyphenols [9], hydrolytic enzymes [10,11], and organic acids, such as glucuronic acid associated with its hepatoprotective effects [12,13] and also for its antimicrobial effects [14]. Ivanišová et al. [9], observed the microbial composition, antioxidant activity, and phytochemicals content (polyphenols, flavonoids, and phenolic acids) in comparison to black tea before it was fermented and the kombucha produced. The antioxidant activity in kombucha was greater than in black tea, in addition to the content of flavonoids. However, theophylline, a compound associated with a strong presence in teas and responsible for inhibiting phosphodiesterase enzymes, was reduced after fermentation.

The production stages of kombucha are not standardized, varying with each producer or company, and different teas and sugar concentrations can be used. That is,

the order and duration of each stage and the amounts of tea, sugar, and inoculum may vary depending on personal and empirical assessments [10].

The microbial and metabolic composition of kombucha varies according to some fermentative parameters, such as the exact composition of SCOBY, the type of tea used [15], the amount of sugar [16,17], the fermentation time [17,18] and the storage time [16].

Table 1 presents different studies with their respective fermentation conditions and their main results.

The main bacteria found in both biofilm and kombucha liquid belong to the group of AAB (gram-negative bacterial acetic acid) and LAB (gram-positive bacterial lactic acid), the two main genera being *Acetobacter* and *Gluconobacter* [21,3]. *Candida krusei*, *Sphingomonas melonis*, *Sphingomonas aquatilis*, *Brevibacillus centrosporus*, and *Gluconobacter oxydans* were the most abundant microorganisms present in kombucha, according to a study by Ivanišová et al. [9].

Chakravorty et al. [22], used traditional culture-independent methods to explore the biofilm bacterial community and reported that *Komagataeibacter sp.* and *Acetobacter sp.* as the main bacterial genera present.

The group of LAB bacteria is generally recognized as safe (GRAS) due to its healthy contribution to the intestinal microbiota of humans. It is believed that its presence in food may have a probiotic effect, due to the production of organic acids, bacteriocins, and other compounds, which also act against pathogenic bacteria.

According to De Roos and Vuyst [6], the presence of some bacteria from the AAB group in fermented drinks, are responsible for quickly and incompletely oxidizing the substrates in organic acids through acidification of the medium. As a result, the oxidation of ethanol to carbon dioxide occurs, through the predominant presence of bacteria of the genus *Acetobacter* and *Gluconobacte* that favor the acidification of the drink, which brings to kombucha a carbonated characteristic typical of soda. The oxidation mechanism of these bacteria produces several compounds, one of which is gluconic acid [23].

Table 1: Initial concentration of tea, sucrose and inoculum, fermentation time and temperature, and main results of different studies

Tea employed and initial concentration (g L ⁻¹)	Initial sucrose concentration (g L ⁻¹)	Inoculum concentration (v/v)	Fermentation time (days)	Fermentation temperature (°C)	Main results	References
Black tea and 1.5	66.47	15%	3.5 - 5	22 - 30	There was no relationship between the reaction rate in the production of kombucha and the independent variables, such as temperature and inoculum concentration.	[19]
Black tea and green tea and 7.5	50	5%	3.75	30	Greater elimination abilities against 2,2-diphenyl-1-picrilhidrazil (DPPH), superoxide anion, and hydroxyl radicals were using green tea. In 10 days and 8 days of storage, the acetic acid and lactic acid bacteria decreased, respectively.	[16]
Black tea and 5	100	10%	18	23 - 27	Antifungal activity from the inhibitory diameter of <i>A. flavus</i> (16.83mm), <i>C. albicans</i> (15.36mm) and <i>M. gypseum</i> (25.06 mm).	[20]
Black tea and 5	30	-	7	22	Antioxidant activity (1.16 mg TEAC mL ⁻¹), total polyphenols (0.42 mg GAE mL ⁻¹), flavonoids (0.13 mg QE mL ⁻¹) and phenolic acids (0.19 mg CAE mL ⁻¹).	[9]
Black tea and green tea and 12	50	3%	10	25	127 phenolic compounds were identified. Greater antioxidant capacity in black tea (65.32%), and greater antibacterial activity in green tea.	[15]
Black tea, green tea and peppermint and 10	20, 50 and 80	2.5%	15 and 20	25	Greater antibacterial activity was against <i>S. aureus</i> , obtained by preparing it with green tea and peppermint in the fermentation time of 21 days and at the levels of 2% and 8%, of the sugar concentrations, respectively.	[17]

3.1 DIFFERENCES IN BIOACTIVITIES IN TYPES OF TEAS

The chemical composition of kombucha can vary according to the type of tea used, with green tea and black tea being the most common. Green tea is obtained from fresh leaves of *Camellia sinensis* L. with catechins being the main polyphenols present. Distinctly, during the manufacture of black tea, the leaves of *Camellia sinensis* L. are subjected to a process that stimulates the activity of polyphenol oxidases and, consequently, the oxidation of catechins and the formation of dimers and polymers known as theaflavins and thearubigins, which are polyphenolic compounds. The different phenolic compounds present directly interfere with the bioactive properties of the kombucha produced [15].

Cardoso et al. [15], obtained an antibacterial activity using green tea of 250 $\mu\text{L mL}^{-1}$, a high value when compared to that obtained using black tea. However, kombucha from black tea fermentation showed a superior antioxidant capacity of 13.59 $\mu\text{mol TE mL}^{-1}$.

In the study by Battikh et al. [24], it was reported that the behavior of the same strain was different about the antimicrobial effect when using green tea and black tea. The fermentation of green tea showed antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* higher and remained constant after the heat treatment. This demonstrates that biologically active components derived from tea, such as enzymes and phenolic compounds, influence the observed antimicrobial activity since when using black tea this activity for the inhibition of each of these microorganisms has reduced.

Jayabalan et al. [7] reviewed other sources of substrates for fermentation such as lemon balm tea, blackberry tea, jasmine tea, oolong tea, sage, thyme, mint teas, among others. In the study by Valiyan et al. [17], antibacterial activity in kombucha prepared with peppermint was greater than in other herbal teas.

According to Filippis et al. [25], the increase in acetic bacteria increases the production of cellulose and, consequently, increases the thickness of the SCOBY (cellulosic film). The nutrients in tea are essential for the growth of microorganisms and the production of the cellulosic film, where Sharma and Bhardwaj [26] reported that fermentation using only water with sugar does not generate a cellulosic film confirming the essential role of tea in fermentation and the generation of SCOBY.

Tea is an essential ingredient for the growth and development of SCOBY, as it contains nutrients that are found in tea leaves, such as nitrogen, caffeine, theanine, and purine and its combination with sugar allows a prosperous development of the colonies present in kombucha [27].

3.2 QUANTITY AND TYPE OF SUGARS

One of the determining factors in the composition and sensory characteristics of the kombucha is related to the sugar content added during the preparation of the tea for fermentation. Ivanišová et al. [9] added 30 g of white sugar to the volume of 1 L of black tea, and at the end of the fermentation process showed antioxidant activity between 1.16 and 2.04 mg TEAC mL⁻¹ and the presence of bioactive compounds, making the kombucha drink an excellent choice for alternative medicine and with numerous benefits for the health of the human body.

Yunirato et al. [20] used the proportion of 6% of white sugar in black tea to prepare the inoculum to assess antifungal activity. Valiyan, Koohsari and Fadavi [17] tested the concentrations of 2.5 to 8% of white sugar in black tea to evaluate the antibacterial activity of kombucha. There was a significant effect of the sugar concentration on the antibacterial activity because sugar is responsible for the metabolism of glucose into fructose by the microorganisms present in the kombucha. The authors cite that for the evaluation of the antibacterial activity tested, the concentration of 8% of sugar was the one that presented the best results.

Other types of sugars as a carbon source have been reported in the literature, such as grape juice [28], milk [29], Salak snake fruits [30], and pear cactus juice [31]. The application of other carbon sources can have different influences on the formation of kombucha compounds. That is, different microorganisms have a preferential sugar in their metabolic route. As SCOBY is composed of several microorganisms, it is suggested that they act in a synchronized manner, each with its speed during fermentation, making available and consuming sugars [3,30,32].

Sun, Li and Chen [33] evaluated the kombucha fermentation with black tea and wheat sprout juice as a substrate and the results showed that the total phenolic and flavonoid contents of the modified kombucha were higher than those of traditional preparations. In this study, it was attributed that the addition of phenolic compounds may come from polyphenols since the SCOBY constituent microorganisms release enzymes

during the evolution of the fermentation, which will degrade the polyphenols into small molecules.

Therefore, the component added as a carbohydrate source interacts individually with SCOBY during fermentation, which may decrease or increase the content of bioactive compounds present in the initial drink. The behavior in the formation of these compounds during the fermentation period is directly related to how the microorganisms present in SCOBY act, influencing the fermentation times that the compound will present its peak concentration. However, the sensory evaluation must be combined with changes in substrates, as the final product can be promising in physical-chemical terms but your taste interferes negatively.

3.3 FERMENTATION TIME AND THE STORAGE PERIOD

The exact mechanism of sucrose fermentation by kombucha has been little reported in the literature, therefore, the knowledge about available fermentation kinetics is limited. The best-understood reaction parameters are the temperature and the concentration of the inoculum. Lončar et al. [19] determined two mathematical models for sucrose fermentation kinetics: Boltzmann's function (alteration of sucrose concentration during fermentation) and reaction rate, both models enabled a better understanding of sucrose transformation. After 10 days of fermentation, it was found that the reaction mixtures contained 2 g L^{-1} of glucose and 3.25 g L^{-1} of fructose, showing that glucose was consumed more quickly than fructose.

In this study, the behavior in the formation of kombucha was also evaluated to the temperature and concentration of the inoculum, with the increase in temperature and concentration there was an increase in the fermentation rate and, consequently, in the concentration of products. In this sense, the ideal fermentation time will depend on the temperature and the inoculum concentration. Studies have reported the ideal fermentation duration of 3 to 5 days, at which time the feed rate reaches its maximum and then decays rapidly [16,34].

Valiyan, Koohsari and Fadavi [17] prepared kombucha drinks with four herbal teas including black tea, green tea, lemon verbena, and peppermint in different concentrations of sugar and at different fermentation times. Changes in sugar concentration, fermentation time, and type of herbal tea have been reported to have significant effects on the *E. coli* inhibition zone. With an increase in sugar concentration and fermentation time, there was an increase in antibacterial activity. The maximum

antibacterial activity against *Bacillus cereus* was achieved with black tea with a 26 mm zone of inhibition of bacteria *S. aureus* at a concentration of 8% sugar and a fermentation time of 21 days.

Kombucha can be stored for a long period without losing its sensory characteristics, making storage time an important parameter associated with its conservation. However, research on changes in the content of probiotics during storage is rare. Fu et al. [16] investigated changes in the content of probiotics in kombucha during storage. When storing the kombucha obtained by fermenting green tea under refrigeration at 4°C, there was a decrease in the growth of acetic acid and lactic acid bacteria after 14 days of storage.

Studies on the increase of bacteria during fermentation, storage, and shelf life must be carried out, not only to ensure food safety but also to produce kombucha as a functional and food-safe product.

From an analysis of the data presented, it is noted that the production process of the kombucha inoculum can vary greatly about the concentration of sugar, type of tea, and other determining factors because it is a drink produced by hand. Thus, there is a gap in the standardization of methods and the fermentation process for industrial scaling and mass marketing due to the numerous benefits to human health mentioned.

4 BENEFITS AND TOXICITY OF CONSUMPTION AND CHALLENGES IN INDUSTRIAL PRODUCTION

The functional drinks sector is a segment of expressive growth in recent years, mainly because it is simpler to add healthy supplements to a drink than to a food [2]. The consumer market is the first parameter to be taken into account consideration for the industrial production of these drinks. In recent years, the trend to seek healthier food lines has reduced the consumption of soft drinks, as consumers have started to look for drinks considered healthier, such as juices and tea. Within this context, kombucha stands out for the reports that are beneficial to human health [34] and for its simple preparation [35]. In the search for natural fermented drink, kombucha is a major challenge for industries [36].

The main health benefits reported are a balance of intestinal flora, reduced cholesterol level [5], antioxidant effects [9,16], and antihyperglycemic effect [37]. It is worth mentioning that many are not yet scientifically proven, and more research is needed to assess whether these benefits exist or not.

Scientific evidence also indicates d-citric acid-1,4-lactone (DSL), a substantial constituent of kombucha, which restricts the activity of the enzyme glucuronidase, which is directly related to cancer [34]. Besides, kombucha can modulate the immune system, reducing oxidative stress, which increases the immune system by containing vitamin C, which is provided by bacteria in the AAB group [38]. However, as it is a homemade drink, there are several limitations to its industrial production.

The limitations arise, precisely because the process is not yet fully controlled and standardized, which generates products with different final characteristics, as well as the microbial and physical-chemical composition. In this sense, the evaluation of fermentative parameters because of specific and beneficial results, can serve as a basis for further studies and, mainly, assist in which operational conditions are best to be produced on a large scale.

4.1 FOOD SAFETY

The spread of kombucha consumption in the world is justified, mainly, by the easy method and without standardization of production. As it is a fermented drink, aspects of food security must be discussed. However, there are a low number of studies that prove the safety of drinking the drink about its benefits, making it a gap for large-scale production.

The possibility of contamination of the drink during its artisanal preparation is high because until reaching a pH close to 4, tea can be contaminated by pathogenic microorganisms, due to the presence of AAB bacteria. Fungal contamination can also occur in Kombucha cultures, especially with *Penicillium* and *Aspergillus*, these are known to cause carcinogenic and toxigenic effects [18]. Besides, some studies have reported lead contamination due to the use of ceramic-based fermentation containers [39]. Most ceramics contain very low levels of lead, which poses no danger to the preparation of tea. However, if the kombucha is fermented for a long time, large amounts of lead can dissolve due to the high concentrations of acetic acid present and metal contamination of the clay used in making the ceramic containers [7]. An alternative proposed by Watawana et al. [18] is to use glass materials for the production and storage of kombucha avoiding the leaching of toxic elements. Therefore, it is important to standardize the process, from fermentation parameters to the materials and components to be used, for less or no contamination.

Although the various beneficial reports of the consumption of kombucha there is evidence of possible toxicity associated with it. According to studies, after consumption, some people experienced diarrhea and nausea [34], suspected liver damage [40], shortness of breath, tightness in the throat, headache, dizziness, and jaundice [41]. Watawana et al. [18] reviewed possible allergic reactions and poor digestion after consumption by people with sensitivity to acids and renal failure. In this context, further studies should be carried out to discuss the safety aspects of kombucha consumption. Also, guidance on the assessment of the safety of AAB for use as microbial food cultures is necessary to make the most of this group of microorganisms.

5 CONCLUSIONS

The spread of artisanal production of the kombucha drink worldwide has been presented as a trend, however, its industrial production is still limited by some factors. The relationship obtained by the word cloud and the articles searched for shows that there is a compatibility between microbiological terms and the need to understand the microbial activity, as well as its composition. There is also a significant presence of AAB bacteria, making it evident the exploration of experimental subjects involving food security.

Given this context, because it is a drink that can be made by hand, it is necessary to disseminate scientific information about its production, to inform the population about the correct preparation conditions, alternative sources of substrate, as well as standardize these conditions. for the industrial process.

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