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Development, microbiological and physicochemical analysis of kombucha-based fermented beverage

Desenvolvimento, análise microbiológica e físico-química de bebida fermentada à base de kombucha

Desarrollo, análisis microbiológico y físico-químico de bebida fermentada a base de kombucha

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Abstract

Fermented kombucha-based beverages have been gaining many adepts around the world because their consumption is associated with beneficial effects on the intestinal microbiota and in some chronic diseases. However, such effects are related to the quality of the drink in terms of microbiological and physicochemical aspects. This study aimed to develop and analyze the microbiological and physicochemical quality of a grape-flavored kombucha fermented drink. This is a quantitative, descriptive, and laboratory study. For the production of the drink, the first fermentation was conducted for five days. Subsequently, the drink was savored simultaneously with the second fermentation with a containing method that guaranteed anaerobic conditions. The beverage was analyzed on the day of filling (Time 0) and on the third day of fermentation (Time 3), regarding the microbiological aspects (total coliforms, presence of *Salmonella* spp., lactic and acetic bacteria) and physicochemical (pH, acidity, ºBrix). It was not identified in any of the total coliform, thermotolerant, and *Salmonella* spp. Regarding lactic acid bacteria, they predominated in Time 3 while acetic bacteria in Time 0. As for the physicochemical parameters, pH values were high during the fermentation process (ranging from 3.5 to 3.78), acidity was constant (7.5%) and a slight reduction was observed for the soluble solids on days 0 and 3 (9.0 and 8.5, respectively). As for microbiological standards, the analyses attested to the satisfactory quality of the drink produced. Regarding the physical-chemical parameters, it was observed that the pH values were above the established by the legislation.

Keywords: Fermentation; Green tea; Black tea.

Resumo

Bebidas fermentadas à base de kombucha vem ganhando muitos adeptos em todo o mundo pelo ato do seu consumo estar associado a efeitos benéficos na microbiota intestinal e em

algumas doenças crônicas. Porém, tais efeitos estão relacionados a qualidade da bebida quanto aos aspectos microbiológicos e físico-químicos. O objetivo deste estudo foi desenvolver e analisar a qualidade microbiológica e físico-química de uma bebida fermentada à base de kombucha de sabor uva. Trata-se de um estudo quantitativo, descritivo e laboratorial. Para produção da bebida, a primeira fermentação foi conduzida por cinco dias. Posteriormente, realizou-se a saborização da bebida concomitante à segunda fermentação com envase que garantiu condições anaeróbicas. A bebida foi analisada no dia do envase (Tempo 0) e no terceiro dia de fermentação (Tempo 3), quanto aos aspectos microbiológicos (coliformes totais, presença de *Salmonella* spp., bactérias láticas e acéticas) e físico-químicos (pH, acidez, ºBrix). Não foi identificado em nenhum dos tempos coliformes totais, termotolerantes e *Salmonella* spp. Com relação às bactérias láticas, elas predominaram no Tempo 3 enquanto as bactérias acéticas no Tempo 0. Quanto aos parâmetros físico-químicos, os valores de pH ficaram elevados durante o processo fermentativo (de 3,5 a 3,78), a acidez esteve constante (7,5%) e foi observada uma ligeira redução no teor de sólidos solúveis nos dias 0 e 3 (9,0 e 8,5, respectivamente). Quanto aos padrões microbiológicos, as análises atestaram a qualidade satisfatória da bebida produzida. Em relação aos parâmetros físico-químicos, observou-se que o valores de pH estavam fora do estabelecido pela legislação.
Palavras-chave: Fermentação; Chá verde; Chá preto.

Resumen

Las bebidas fermentadas a base de kombucha han ido ganando adeptos en todo el mundo porque su consumo está asociado a efectos beneficiosos sobre la microbiota intestinal y en algunas enfermedades crónicas. Sin embargo, dichos efectos están relacionados con la calidad de la bebida en términos de aspectos microbiológicos y físico-químicos. El objetivo de este estudio fue desarrollar y analizar la calidad microbiológica y físico-química de una bebida fermentada de kombucha con sabor a uva. Se trata de un estudio cuantitativo, descriptivo y de laboratorio. Para la elaboración de la bebida se realizó la primera fermentación durante cinco días. Posteriormente, la bebida se saboreó concurrentemente con la segunda fermentación con relleno que garantizaba condiciones anaeróbicas. La bebida se analizó el día de llenado (Tiempo 0) y el tercer día de fermentación (Tiempo 3), en cuanto a los aspectos microbiológicos (coliformes totales, presencia de *Salmonella* spp., Bacterias lácticas y acéticas) y fisicoquímicos (pH, acidez, ºBrix). No se identificó en ninguno de los coliformes totales, termotolerantes y *Salmonella* spp. En cuanto a las bacterias lácticas, predominaron en el Tiempo 3 y las acéticas en el Tiempo 0. En cuanto a los parámetros físico-químicos, los

valores de pH fueron altos durante el proceso de fermentación (3,5 - 3,78), la acidez fue constante (7.5%) y se observó una ligera reducción en los tiempos (9.0 - 8.5) para el contenido de sólidos solubles. En cuanto a los estándares microbiológicos, los análisis dieron fe de la calidad de la bebida producida. En cuanto a los parámetros físico-químicos, se observó que los valores de pH se encontraban fuera de los establecidos por la legislación.
Palabras clave: Fermentación; Té verde; Té negro.

1. Introduction

The consumption of foods and beverages that have additional beneficial health effects has been increasingly frequent in the world population. Foods with these characteristics are called functional, being defined as those that exhibit properties beyond the nutritional function relevant to any food, derived from the presence of bioactive compounds that, when consumed as part of a usual diet have an antimicrobial antioxidant function, anti-inflammatory, therefore, help prevention in the occurrence of chronic non-communicable diseases, in intestinal and immunological modulation (Philippi, 2018; Costa & Rosa, 2016; Mahan & Raymond, 2019).

Among the bioactive compounds present in these foods, stands out the phenolic compounds, as one of the most abundant classes, and are classified into; flavonoids (anthocyanins, proanthocyanidins, flavones, flavonoids, isoflavones, flavanones, flavans, flavan-3-ols, among others) and non-flavonoids (ellagitannins, stilbenes, lignans, gallotannins), carotenoids, alkaloids, organosulfur compounds, as well as compounds and microorganisms with prebiotic and probiotic action (Ávila et al., 2018).

Fermented beverages, rich in bacteria and yeast, may be included in the functional food group, some with probiotic properties acting a fundamental role in the maintenance of the intestinal microbiota. In this context, kombucha is an example of a fermented beverage rich in prebiotics and probiotics that limit the proliferation of pathogenic bacteria in the intestinal environment (Faridah**,** Diana, Helmi, Sami, & Mudliana, 2013).

Kombucha is a millenary drink of Asian origin, fermented by a consortium of acetic bacteria and yeasts, which has gained many adepts worldwide and as consequence, its effects on the body have been widely studied (Bruschi, Sousa & Modesto, 2018; Faridah et al., 2013; Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014).

Some studies show that antimicrobial, anti-inflammatory, detoxifying, antioxidant, and probiotic action is some of the already proven effects of kombucha, besides acting directly on reactions caused by chronic diseases and also known as underlying diseases, as well as in controlling the differentiation and proliferation of carcinogenic cells (Battikh**,** Bakhrouf, & Ammar, 2012; Fu, Yan, Cao, Xie, & Lin**,** 2014; Villarreal-Soto, Beaufort, Bouajila, Souchard, & Taillandier, 2018).

Given all the proven effects, the analysis of microbiological and physicochemical characteristics are relevant to ensure the quality of this beverage, since the production process is usually artisanal which can present a risk to the health of the consumer. Thus, this study aimed to develop and analyze the microbiological and physicochemical quality of a fermented drink based on grape-flavored kombucha.

2. Material and Methods

This is a quantitative, descriptive, and laboratory study (Dalfovo, Lana, & Silveira, 2008). The experiments were carried out in the Laboratories of Gastronomy and Dietary Techniques of the University Center of Technology and Sciences (Beverage Elaboration), in partnership with the Microbiology Laboratory of the State University of Southwest Bahia, where microbiological and physicochemical analyses were performed.

For the preparation of the beverage, the ingredients and their proportions were established using preliminary tests and are listed in Table 1.

Table 1 - Ingredients and proportions used for the production of the fermented beverage.

Ingredients	Quantity
Scoby	80 g
Starter tea (Matured Kombucha)	150 mL
Green Tea	6 g
Black tea	2 g
Filtered and boiled water	1350 mL
White organic sugar	80 g
Whole grape juice	160 mL

Source: Authors.

The symbiotic consortium of microorganisms, called scoby or "mother kombucha", as well as starter tea and herbs (mate tea and black tea) used in the preparation of the beverage, were acquired in the online market, while the other ingredients were obtained in the local market of Vitória da Conquista, Bahia, Brazil.

2.1 Development of the beverage

The preparation of the fermented beverage was initiated by the elaboration of an infusion of black and green tea. Then, 150 mL of water was heated to 80 ºC followed by the addition of black and green tea herbs, which remained in infusion for 9 minutes. After this time, the tea was filtered and the rest of the water (1200 mL) and sugar was added to it. When the tea reached the temperature of 25 ºC, it was packed in a properly sterilized glass container, plus 150 mL of starter tea (matured kombucha) and the scoby. The container was covered with sterile voal tissue, fastened with elastic, and stored in a dry and ventilated place for 5 days for the development of the fermentation process (first fermentation).

After this period, the second fermentation started concomitantly with the flavoring of the fungus broth and whole grape juice. For this purpose, glass bottles with a capacity of 250 mL and plastic caps were used for containing and sealing, respectively. The grape juice (80 mL) and kombucha fungus broth (160 mL) produced in the first fermentation (Filling process - Time 0) were added into the container. The bottles were stored at room temperature (23 to 26 ºC), for three days, a period determined for the second fermentation (Time 3). After that, the bottles were refrigerated at 5 ºC, which remained until the moment of the analysis, a period of 24 hours (Amarasinghe; Weerakkody & Waisundara, 2018).

2.2 Microbiological and physicochemical analyses

The microbiological analyses of total and thermotolerant coliforms, *Salmonella* spp., lactic and acetic bacteria counting, were performed using the protocol described by Silva et al. (2017). The physicochemical analyses evaluated: pH, Brix (soluble solids), and total acidity, according to instructions from the Adolfo Lutz Institute (2008). All analyses were performed in triplicate using samples of the beverage on the day of the sample containing (Time 0) and after 3 days of beverage fermentation (Time 3).

Concerning the microbiological analyses, the beverages were proceeded with serial dilution, in which 25 mL of the beverage were transferred into an Erlenmeyer containing 225 mL of 0.01% peptone water properly sterilized. Then, the sample was homogenized, resulting in the first dilution 10^{-1}. To make up the dilution of 10^{-2}, 1 mL of 10^{-1} was transferred into a

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tube containing 9 mL of peptone water, and the procedure was repeated until dilution of 10^{-5} (Silva et al., 2017).

Regarding the quantification of total and thermotolerant coliforms, the multiple tube technique was adopted. The first stage was the presumptive test, in which aliquots of the first three dilutions were transferred into tubes containing lauryl sulfate tryptic broth, followed by incubation at 35 ºC for 48h. Subsequently, tubes with a positive result in the presumptive test (turbidity of medium and formation of gas) were identified and aliquots were transferred into Bright green broth, for total coliforms test, and into tubes containing EC broth, for thermotolerant tests, followed by incubation at 35 ºC and 44.5 ºC, respectively, for 48h. Positive results were evidenced by the turbidity of medium and formation of gas. The results were presented in Most Probable Number per mL of the beverage (MPN/mL).

For *Salmonella* spp. analysis, the method is separated into five stages: pre-enrichment in non-selective medium (incubation of the first dilution 35 ºC/24h); selective enrichment (Selenium and Tetrathionate medium); Selective plating (XLD- Deoxycholate-lysine-xylose); preliminary confirmation of colonies typical of *Salmonella* spp. in TSI/LIA agar (Triple Sugar Iron and Iron Lysine Agar, respectively); and biochemical tests (glucose, tryptone, and lactose). The results were expressed in the absence or presence of *Salmonella* spp.

For counting of acetic bacteria, pour-plating with overlayer technique was adopted using two media (First layer: 2 g of 0.5% agar, 2 mL of acetic acid, 8 mL of ethanol; Second layer: 4 g of yeast extract, 4 g of glucose, 4 g of acetic acid, 4 g of peptone and 16 mL of ethanol). One milliliter of each dilution was packed in sterile plates, then, the first medium was added and after solidification, the second half was added. The plates were incubated in a greenhouse with a controlled temperature at 35 ºC for 7 days, after this period the number of colonies formed was counted. The results were expressed in CFU/mL.

Concerning the quantification of lactic bacteria, the pour-plating with overlayer technique in the MRS medium was used. After inoculation, the plates were kept in incubation with a controlled temperature at 35 ºC for 48 hours, after this period the counting of the colonies was performed and the results were expressed in CFU/mL (Silva et al., 2017).

In regard to the physicochemical analyses, acidity was determined by titration, using sodium hydroxide (NaOH) 0.1%, and phenolphthalein as an indicator. For pH analysis, 100 mL of the beverage was packed in a beaker and analyzed in pHmeter (Micronal®), previously calibrated with a standard solution. For the analysis of Brix- soluble solids, a drop of the sample collected with a sterile pipette was placed directly in the lens of the refractometer

(Quimis®) properly sterilized. The results were shown by the mean and standard deviation of data.

3. Results and Discussion

The data obtained through the analyses performed in the samples of the kombucha, at Time 0 (day of the filling process) and three days after time 3, are shown in Table 2 (microbiological analysis) and Table 3 (physicochemical parameters).

Table 2 - Results of counting of microbiological markers in kombucha.

Analyzed Parameters	Time 0	Time 3
Total coliforms	0 MPN/mL	0 MPN/mL
Thermotolerant coliforms	0 MPN/mL	0 MPN/mL
Salmonella spp.	Absent	Absent
Lactic bacteria	>25 CFU/mL	$1,1 \times 10^3$ CFU/mL
Acetic bacteria	$3,3 \times 10^2$ CFU/mL	>25 CFU/mL

Source: Authors.

Through the analysis of total coliforms, thermotolerant, and *Salmonella* spp., it can be affirmed that the developed beverage has satisfactory microbiological quality, regarding both analyses with the absence of such microorganisms. The total coliforms found are proportional to those described by Santos (2017) in a study of obtaining and characterizing black tea kombucha.

High total coliform counts are associated with failures, especially concerning the hygiene of raw materials, environment, utensils, and the handler as well. These microorganisms reduce the quality of the final product, accelerating its deterioration, and consequently reducing the shelf-life (Franco & Landgraf, 2008; Jay, 2009).

The analysis of *Salmonella* spp. in artisanal beverages is of fundamental importance due to the risk of contamination by these microorganisms, which are capable of causing pathologies triggered by their ingestion and may lead the consumer to death (Franco & Landgraf, 2008; Jay, 2009; Forsythe, 2013).

In fermented beverages, the sum of the application of good manufacturing practices, the choice of ingredients with proven quality, and acid pH ensure the safety of the beverage from a microbiological point of view (Machado; Dutra & Pinto, 2015). Besides, the fermentative process with a predominant concentration of acetic bacteria metabolizes sucrose, used as the substrate in tea, generating the release of organic acids, such as glucuronic and acetic acid, which generates a selective inhibition of pathogenic microorganisms in the medium, which can be proven by the obtained results (Tondo et al., 2015).

When analyzing the lactic and acetic bacteria count, an inversion was observed in the values found of these microorganisms with the evolution of fermentation time, where, on day 0, high counts of acetic bacteria prevailed, while the population of lactic bacteria was less than 25 CFU/mL. On day 3, the opposite was verified.

It is known that acetic bacteria tolerate media with more acidic pH (3.5%), and therefore create a mechanism of competition with lactic bacteria. The competition between these bacteria gives the medium more acidic characteristics due to the transformation of the substrates during fermentation in the beverage favoring the proliferation of acetic bacteria, and the mechanism of competition between them causes the beverage to be slightly carbonated, because of the release of CO_2 from the enzymatic cascade that occurs in the process of hydrolysis of sucrose used in the beverage (Forsythe, 2013).

The results of the physicochemical parameters analyzed are shown in Table 3. The pH values showed a slight increase in this parameter when comparing to the day of the filling process and on the third day. According to regulation No. 103, September 20, 2018, a pH between 2.5 and 3.5 is considered acceptable for these beverages, which allows us to affirm that the pH of the third day is above the recommended. This value can be explained by the short fermentation period (3 days), considering that Schroeder et al. (2019) developed a fermented beverage with kombucha from acerola residue and observing that the pH of the beverage varied from 3.08 to 2.55 in 7 days of fermentation.

Table 3 - Results of physicochemical analyses of kombucha.

Analyzed Parameters	Day 0	Day 3
pH	3,5±0,01*	3,78±0,03*
Acidity (% tartaric acid)	7,5%±0,01*	7,5%±0,01*
ºBrix	9,0±0,04*	8,5±0,07*

* Standard deviation estimate. Source: Authors.

In a work carried out by Bruini et al. (2019) on physicochemical and microbiological aspects in the kombucha manufacturing process, a pH of 3.29 was found, meeting the limit established by the MAPA regulation. Santos (2017) also analyzed the pH of the beverage fermented by kombucha and black tea in the first fermentation, which occurred in 25 days at room temperature. The pH found was 3.98, which exceeds the limits established by the aforementioned regulation (103/2018).

Paludo (2017) presented a study about the development and characterization of kombucha obtained from green tea and yerba mate extract. In this work, the yerba mate beverage presented initial pH of 4.7 and in seven days of fermentation and detecting a pH of 3.10, while in the green tea kombucha, the initial pH value was 4.10 and 2.81 after 7 days of fermentation. These results are explained by the type of herb that was used in each infusion because, as reported in the author, the procedure to obtain a similar pH would be taken from the samples at different times. The results evidenced in this study are in accordance with the legislation.

Concerning the acidity index, according to regulation Nº. 103, September 20, 2018, (Brasil, 2018). The minimum acceptable titratable acidity is 6.0%, which allows us to confirm that the beverage in this study is within the standards established by the legislation. In a study evaluating the quality of kombucha, Bruini et al. (2019) found that the acidity in normal solution was 1.023%. Also, Santos (2017) obtained a value of 1.067 (% acetic acid) in the analysis of total acidity.

In regard to the concentration of soluble solids, expressed in ºBrix, a reduction in concentration was evidenced over the fermentation time. This fact is expected since microorganisms use sugars as an energy source during the fermentation process, thus culminating in their reduction.

4. Final Considerations

The beverage produced showed satisfactory results regarding microbiological quality and most of the physicochemical aspects analyzed were in accordance with current legislation, except for the pH of the beverages that were expected values below 3.5, but this value may be due to the short fermentation period. Besides, the results obtained can be used as a reference in new studies for possible standardization of this beverage, since the constant feeding of the inoculum results in continuous growth.

Finally, it is suggested to evaluate the product in a longer fermentation time to ensure that the physicochemical aspects are within the parameters provided by the legislation, and the performance of sensorial analyses to evaluate the acceptability of the beverage and its potential for commercialization.

References

Amarasinghe, H., Weerakkody, N. S., & Waisundara, V. Y. (2018). Evaluation of physicochemical properties and antioxidant activities of kombucha "Tea Fungus" during extended periods of fermentation. *Food science & nutrition*, *6*(3), 659-665.

Ávila, S., Barão, F. D., da Silva Gonçalves, L., dos Santos, M. A., de Carvalho Furtado, C., & de Souza, C. B. (2018). Compostos bioativos presentes nos chás verde e preto. *UNILUS Ensino e Pesquisa*, *14*(37), 47-57.

Battikh, H., Bakhrouf, A., & Ammar, E. (2012). Antimicrobial effect of Kombucha analogues. *LWT-Food Science and Technology*, *47*(1), 71-77.

Brasil. Ministério da Agricultura Pecuária e Abastecimento/Secretaria de Defesa Agropecuária. (2018). Portaria Nº 103, de 20 de setembro de 2018. Padrão de Identidade e Qualidade de Kombucha. *Diário Oficial da União*.

Bruini, B. (2019). Aspectos físico-químicos e microbiológicos no processo de fabricação da Kombucha. *Revista Engenho*, *11*(1), 48-67.

Bruschi, J. S., Sousa, R. C. S., & Modesto, K. R. (2018). O ressurgimento do chá de kombucha. *Revista de Iniciação Científica e Extensão*, *1*(Esp), 162-168.

Costa, N. M. B, & Rosa, C. O. B. (2016). *Alimentos funcionais: componentes bioativos e efeitos fisiológicos*. (2a ed.), Rio de Janeiro: Editora Rubio.

Dalfovo, M. S., Lana, R. A., & Silveira, A. (2008). Métodos quantitativos e qualitativos: um resgate teórico. *Revista interdisciplinar científica aplicada*, *2*(3), 1-13.

Faridah, F., Diana, S., Helmi, H., Sami, M., & Mudliana, M. (2013). Effect of sugar concentrations on bacterial cellulose production as cellulose membrane in mixture liquid medium and material properties analysis. In *ASEAN/Asian Academic Society international conference proceeding series*.

Forsythe, S. J. (2013). *Microbiologia da segurança alimentar*. (2a ed.) Porto Alegre: Editora Artmed.

Franco, B. D. G. M. & Landgraf, M. (2008). *Microbiologia dos Alimentos*. São Paulo: Editora Atheneu .

Fu, C., Yan, F., Cao, Z., Xie, F., & Lin, J. (2014). Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage. *Food Science and Technology*, *34*(1), 123-126.

I. A. L. (2008). *Normas Analíticas do Instituto Adolfo Lutz. Métodos físico-químicos para análises de alimentos*. (4a ed.), 1020 p.

Jay, J. M. (2009). *Microbiologia de alimentos*. (6a ed.), Porto Alegre: Editora Artmed.

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.

Machado, R. L. P., Dutra, A. de S., & Pinto, M. S. V. (2015). *Boas práticas de fabricação (BPF)*. Rio de Janeiro. Embrapa.

Mahan, L. K., Raymond, J. L. (2019). *Krause alimentos, nutrição e dietoterapia*. (14a ed.), Rio de Janeiro: Editora Elsevier.

Paludo, N. (2017). *Desenvolvimento e caracterização de kombucha obtida a partir de chá verde e extrato de erva-mate: processo artesanal e escala laboratorial*. Trabalho de Conclusão de Curso. Universidade Federal do Rio Grande do Sul - UFRG, Brasil.

Philippi, S. T.(2018). *Pirâmide dos alimentos: fundamentos básicos da nutrição*. (3a ed.), São Paulo: Editora Manole.

Santos, R. C. (2017). *Obtenção e caracterização de kombucha de chá preto*. Tese de Doutorado, Universidade Federal de Minas Gerais - UFMG, Brasil.

Silva, N., Junqueira, V. C. A., Silveira, N. F. A., Taniwaki, M. H., Gomes, R. A. R., & Okazaki, M. M. *Manual de métodos de análise microbiológica de alimentos e água*. (5a ed.), São Paulo: Editora Blucher, 2017.

Tondo, E. C., Casarin, L. S., Oliveira, A. B., Martello, L., da Silva Jr, E. A., & Gelli, D. (2015). Avanços da segurança de alimentos no Brasil. *Vigilância Sanitária em Debate: Sociedade, Ciência & Tecnologia*, *3*(2), 122-130.

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.

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