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Development, characterization and antioxidant activity of kombuchas of cerrado's fruits

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ABSTRACT. Kombucha is a fermented beverage with an acid flavor, prepared from sweetened *Camellia sinensis* tea and a symbiotic culture of bacteria and yeasts, which are responsible for promoting the fermentative transformation. The Brazilian legislation allows the addition of several ingredients and considering the local biome, the objective of this work was to develop, characterize and evaluate kombuchas of green tea with the addition of Cerrado's fruits: seriguela and guavira. The kombuchas of green tea, seriguela and guavira were fermented for 14 days, characterized and subjected to microbiological analysis, determination of phenolic compounds and antioxidant activity by ABTS and DPPH methods. The microbiological, pH and alcohol content standards required by the legislation were achieved. The highest content of phenolic compounds was found in the kombucha of green tea, followed by kombucha of guavira and kombucha of seriguela. The kombucha of guavira showed the highest antioxidant capacity, followed by the kombucha of green tea and the kombucha of seriguela, in both methods. The results demonstrate the potential production of bioactive compounds in kombuchas added to Cerrado's fruits.

Keywords: fruit's kombucha; fermented beverages; organic acids.

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Introduction

Kombucha is a traditional drink from China, made from the fermentation of sweetened black or green tea leaves (*Camellia sinensis*). This process produces a fizzy, refreshing drink with a slightly sweet and acidic taste. A symbiotic system made up of bacteria and yeasts (SCOBY - Symbiotic Culture of Bacteria and Yeasts) is the agent responsible for fermentation (Yildiz et al., 2020).

In general, yeasts transform the sucrose present in the drink into ethyl alcohol using the invertase enzyme. Acetic bacteria then use the alcohol produced by the yeast to produce acetic acid. In addition to these compounds, kombucha fermentation is capable of producing and enhancing numerous other substances, such as polyphenols, organic acids, vitamins, dietary fibers, essential elements (Cu, Fe, Mn, Ni, Zn), hydrolytic enzymes and amino acids (Miranda et al., 2016).

The characteristics of kombucha are influenced by a number of factors, such as the fermentation time and temperature, the type and origin of the tea, the use of additional raw materials and the micro-organisms present in the symbiotic culture (SCOBY). Fermentation usually lasts between 7 and 21 days. A long fermentation period alters the sensory taste of the drink, as there is a higher production of acetic acid. Another product affected by the fermentation period is the SCOBY. Cellulose fibers attached to microorganisms tend to be produced in greater quantities in long fermentation (Leonarski et al., 2021; Emiljanowicz et al., 2019).

However, the health benefits related to kombucha consumption are reported in different types of beverage and production conditions. The consumption of kombucha is able to promote the inhibition of cancer cells, the balance of intestinal microflora, the reduction of atherosclerosis, inflammatory problems, blood pressure and cholesterol levels, the detoxification of the blood, the alleviation of symptoms of rheumatism and arthritis, as well as having antimicrobial, antioxidant and hepatoprotective effects. Some studies have also investigated the association of kombucha and SCOBY with animal feed and have obtained growth-promoting effect in broiler chickens (Afsharmanesh & Sadagh, 2013), increase of plasma HDL levels and reduction of plasma levels of cholesterol, LDL, uricacid, AST, and ALT in broiler chickens (Salehi et al., 2021) and antioxidant activity, anti-metabolic and anti-inflammatory activity in relieving metabolic disorders and inflammatory diseases in vitro and in vivo (Permatasari et al., 2022).

Several studies have shown the addition of other ingredients or even alternatives to green and black tea, resulting in beverages with new properties. Table 1 shows some of the studies carried out using alternative raw materials or those added to traditional teas and their fermentation conditions.

Table 1. Raw materials and fermentation conditions for alternative kombuchas.

Raw-material	Fermentation time	Temperature	Reference
Sunflower artichoke extract	21 days	28°C	Malbaša et al. (2001)
Lemon balm	7 days	28°C	Velićanski et al. (2014)
Cactus fruit (*Opuntia ficus-indica*)	12 days	30°C	Ayed & Hamdi (2015)
Coffee fruit[1]	9 days	28°C	Essawet et al. (2015)
Grape	12 days	30°C	Ayed et al. (2016)
Snake fruit	14 days	28°C	Ifadah et al. (2019)
Asian mustard	14 days	25°C	Rahmani et al. (2019)
Goji berry	2 days	28°C	Tamer & Abuduaibifu (2019)
Rice and barley	12 days	28°C	Ahmed et al. (2020)
Black Carrot[1]	12 days	30°C	Yildiz et al. (2020)
Soursop	21 days	28°C	Tan et al. (2020)
Acerola residue	15 days	30°C	Leonarski et al. (2021)
Mushrooms (*Coriolus versicolor* e *Lentinus edodes*)	11 days	24°C	Sknepnek et al. (2021)
Coffee	8 days	23°C	Bueno et al. (2021)
Seaweed (*Porphyra dentata*)	14 days	25°C	Aung & Eun (2021)

[1]Studies in which the raw material was not used pure but added to green or black tea. Author.

Following the trend of adding fruit to kombucha brewing, seeking new or better properties in the final drink, the aim of this study was to develop, characterize and evaluate the antioxidant activity of kombuchas brewed with green tea (*Camellia sinensis*) and fruits from Cerrado (local biome) - seriguela (*Spondias purpurea*) and guavira (*Campomonesia adamantium*).

Materials and methods

Materials

The SCOBYs and the fermented kombucha used to produce the new kombuchas were kindly provided by an artisan producer in the region of Dourados, Mato Grosso do Sul, Brazil. The fermented kombucha provided was prepared using green tea and fermented for 21 days at 25°C, resulting in a drink with a microbiological count of 10^6 CFU mL^{-1} of total mesophilic bacteria, 10^6 CFU mL^{-1} of lactic acid bacteria, 10^5 CFU mL^{-1} of acetic acid bacteria and 10^7 CFU mL^{-1} of yeasts. This count was carried out in order to obtain control parameters for the preparation of the drinks developed in this study.

The fruits of seriguela (*Spondias purpurea*) and guavira (*Campomonesia adamantium*), imported green tea (*Camellia sinensis*) and sucrose were obtained from local shops in the city of Dourados-MS. The fruits were cleaned under running water and then immersed in sodium hypochlorite diluted in water (1:50) for 15 minutes. After sanitizing, the fruit was pulped manually using sieves. The pulps were frozen in an ultrafreezer at -80°C until analysis.

Production of kombuchas

Kombuchas were produced in the Molecular Biology Laboratory of the Faculty of Biological and Environmental Sciences at the Federal University of Grande Dourados, according to the methodology of Jafari et al. (2020) with adaptations. The infusions were made for 5 min using 8 g L^{-1} of green tea in water boiled at 100°C and cooled to 80°C. After cooling the infusions, three different formulations were prepared: K-Green tea (900 mL of imported green tea infusion + 100 mL of fermented kombucha + 60 g of sucrose + one unit of SCOBY); K-Seriguela (800 mL of imported green tea infusion + 100 mL of seriguela pulp + 100 mL of fermented kombucha + 60 g of sucrose + one unit of SCOBY) and K-Guavira (800 mL of imported green tea infusion + 100 mL of guavira pulp + 100 mL of fermented kombucha + 60 g of sucrose + one unit of SCOBY).

Drinks were packed in glass containers sealed with paper napkins secured with rubber bands and stored at room temperature and away from light to allow fermentation to take place. After 14 days, the fermented drinks were filtered and stored in plastic containers under refrigeration (5°C) until analysis. The drinks were not subjected to the addition of carbon dioxide (CO_2).

Microbiological analysis

Microbiological analyses for lactic acid bacteria, total mesophilic bacteria, acetic acid bacteria and molds and yeasts were carried out using serial dilutions in peptone water, the microdrop technique and plate counts. The culture media used were MRS agar (Merck MRS broth and 15 g mL^{-1} bacteriological agar) for counting lactic acid bacteria, Merck nutrient agar (28 g L^{-1}) for total bacteria, GYC (50 g L^{-1} dextrose, 10 g L^{-1} yeast extract, 20 g L^{-1} bacteriological agar, 5 g L^{-1} calcium carbonate, 70 mL L^{-1} ethanol) for acetic bacteria and YPD (10 g L^{-1} yeast extract, 20 g L^{-1} dextrose, 20 g L^{-1} peptone, 20 g L^{-1} bacteriological agar) for molds and yeasts (Cardoso et al., 2020; Taheur et al., 2020).

Salmonella testing was carried out according to the method proposed by the Association of Official Analytical Chemists (Association of Official Analytical [AOAC], 2016). The total thermotolerant coliform count was carried out according to the method proposed by the Ministry of Agriculture, Livestock and Supply (Brasil, 2019c).

Characterization of the drinks

Volatile acidity was determined using a titrimetric method, separating the volatile acids using steam drag. The volatile acidity values were presented in milliequivalents per liter (mEq L^{-1}), for comparison with the legislation (Brasil, 2005).

The definite alcoholic strength was determined by separating the alcohol by distillation and quantifying it according to relative density, in agreement with the instructions in the Operational Manual for Beverages and Vinegars (Brasil, 2005). The pH values were obtained using a benchtop digital pH meter (Medidor Lab). The soluble solids content, expressed in °Brix, was determined using a benchtop refractometer (Refratomer 704030, model 2waj, Brazil).

Total phenolic compounds

Extract preparation

The extracts were prepared according to Asami et al. (2003) with adaptations. An extracting solution of acetone, acetic acid and water (70:0.5:29,5 v/v/v) was used, in a ratio of 10 mL of sample to 20 mL of solution (10:20 v/v). The mixture was centrifuged (Hettich, Germany) at 1500 rpm for 10 min, and the supernatant was separated and considered an extract.

Determination of total phenolic compounds using the Folin-Ciocalteau method

Total phenolic compounds were determined according to the method of Singleton et al. (1999). A calibration curve was constructed using different concentrations of gallic acid (100 to 1000 μg mL^{-1}) as a standard.

In the tubes, 50 μL of extract, 750 μL of 7% sodium carbonate, 250 μL of Folin-Ciocalteau reagent and 3 mL of distilled water were added. The mixtures were shaken and a further 950 μL of distilled water was added. The tubes were then left to stand for 2 hours in a dark environment. After resting, the absorbance readings were taken on a spectrophotometer (Biochorom, UK) at 765 nm, using distilled water as a blank. The procedure was carried out for each sample in triplicate.

The content of total phenolic compounds was determined by interpolating the absorbance of the samples and the standard curve. The results were presented in milligram equivalents of gallic acid per milliliter of sample (mg GAE per mL of sample).

Antioxidant activity

Preparation of extracts

10 mL of the drink sample was measured, added to 40 mL of methanol (50%) and left to stand for 60 min. The mixture was centrifuged (Hettich, Germany) at 1500 rpm for 20 min and the supernatant was separated into a volumetric flask.

40 mL of acetone (70%) was added to the residue obtained during centrifugation and left to stand for a further 60 min. The mixture was centrifuged at 1500 rpm for 20 min. The supernatant was added to the flask containing the previous supernatant and the volume was adjusted to 100 mL with distilled water (Rufino et al., 2007a).

Determination of antioxidant activity using the ABTS method

The determination of antioxidant activity using the ABTS•+ radical capture method (2,2'-azinobis-(3 ethylbenzothiazoline-6-sulfonic acid)) was carried out according to Rufino et al. (2007a). A curve was constructed using different concentrations of Trolox solution (100 μM to 2000 μM).

Different dilutions of the beverage extracts were prepared for each sample. In test tubes, 30 μL of each dilution and 3 mL of ABTS•+ radical solution was added. The mixtures were homogenized in a tube shaker and left to stand for 6 min, protected from light. After resting, the absorbance readings were taken on a spectrophotometer at 734 nm, using ethyl alcohol as a blank. The results were obtained by linear regression of the standard curve and presented in μM of trolox per mL of extract.

Determination of antioxidant activity using the DPPH method

The determination of antioxidant activity using the DPPH• (1,1-diphenyl-2-picrylhydrazine) method was carried out according to Rufino et al. (2007b). A standard curve was constructed using different concentrations of DPPH solution (10 to 60 μM).

Different dilutions of the drink extracts were prepared for each formulation. 0.1 mL of each dilution was added to 3.9 mL of DPPH• radical solution in test tubes and homogenized in a tube shaker. After 30 minutes at rest, protected from light, the absorbance readings were taken on a spectrophotometer at 515 nm, using methyl alcohol as a blank.

The results were obtained by linear regression of the standard curve, generating the EC_{50} index, which is the amount of sample needed to reduce the initial concentration of the DPPH• radical by 50%. The indices were presented in milliliters of extract per gram of DPPH (mL extract/g DPPH).

Statistical analysis

The results obtained were expressed as triplicate means and standard deviation. The values were subjected to analysis of variance (ANOVA) and Tukey's mean comparison test ($p \leq 0,05$) using GraphPad Prism® 8 software.

Results and discussion

Table 2 shows the values obtained for the characterization analyses of the beverages produced.

Table 2. Characterization of green tea, seriguela and guavira kombuchas.

		K-Green tea	K-Seriguela	K-Guavira
pH	Day 0	3.21 ^{aA} ± 0.01	3.00 ^{cA} ± 0.01	3.16 ^{bA} ± 0.00
	Day 14	3.12 ^{aB} ± 0.02	2.78 ^{cB} ± 0.00	2.96 ^{bB} ± 0.05
Soluble solids (°Brix)	Day 0	6.80 ^{aA} ± 0.00	6.80 ^{aA} ± 0.00	6.80 ^{aA} ± 0.00
	Day 14	5.20 ^{bB} ± 0.00	3.20 ^{cB} ± 0.00	5.40 ^{aB} ± 0.00
Volatile acidity (mEq L^{-1})		16.52 ^{c} ± 0.00	213.15 ^{a} ± 0.00	47.92 ^{b} ± 0.00
Alcoholic (%v/v)		0.08 ^{c} ± 0.00	0.62 ^{a} ± 0.00	0.26 ^{b} ± 0.00

Different lowercase letters on the same line indicate a significant difference in Tukey's test ($p < 0.05$); Different uppercase letters indicate a significant difference only between the same drink between days 0 and 14 in Tukey's test ($p < 0.05$); K-Green tea = green tea kombucha; K-Seriguela = seriguela kombucha; K-guavira = guavira kombucha.

Normative Instruction No. 41 of September 17, 2019 establishes the standard of identity and quality of kombucha in Brazil. The minimum and maximum pH parameters are 2.5 and 4.2, respectively. According to the pH results obtained, the kombuchas produced comply with the standards of current legislation (Brasil, 2019a).

All the samples showed a drop in pH after fermentation, with a reduction of between 0.09 and 0.22. The seriguela kombucha showed the greatest variation in values between the fermentation period and the lowest pH, which may be related to the high amount of carbohydrates in the fruit pulp (from 11.8 to 21.54 g 100 g^{-1}), which increases the fermentation rate and consequently reduces the pH. Green tea kombucha, on the other hand, showed the lowest variation and the highest pH value, possibly due to the low concentration of carbohydrates in green tea, since it is in leaf form (Food and Agriculture Organization of the United Nations & World Health Organization [FAO/WHO], 2001).

All the pH values differed between the different drinks. The values of the same drink at the different times (initial and final) also differed significantly. Bueno et al. (2021) made coffee kombucha and obtained pH values of 3.62 ± 0.03 on day 0 and 3.60 ± 0.04 on day 15. The pH values of beverages are directly linked to acidity. Consequently, seriguela kombucha had the highest volatile acidity (1.29 ± 0.00 g 100 mL^{-1}) and green tea kombucha the lowest, correlating with pH values and the presence or absence of carbohydrates in the raw material. All the volatile acidity averages for all the formulations differed significantly from each other.

When the volatile acidity values are compared to the legislation, only the guavira kombucha (47.92 ± 0.00 mEq L^{-1}) is within the standards, which state that volatile acidity should be between 30 and 130 mEq L^{-1}. The green tea (16.52 ± 0.00 mEq L^{-1}) and seriguela (213.15 ± 0.00 mEq L^{-1}) kombuchas were below and above the current standards, respectively (Brasil, 2019a). The composition of the added fruit may be responsible for the differences in volatile acidity between the samples. However, compliance with the parameters of the legislation could be achieved by adjusting the fermentation time and constantly controlling the pH.

The results obtained are close to other studies of fruit kombuchas. Tamer & Abuduaibifu (2019), obtained acidity of 38.00 ± mEq L^{-1} and 42.96 ± 0.01 mEq L^{-1} for black and red goji berry kombuchas fermented for 11 days. Ayed et al. (2016) analyzed grape kombucha fermented for 12 days and obtained an acidity of 104.2 ± 3.2 mEq L^{-1}.

The increase in acidity and drop in pH are related to the production of organic acids during the fermentation process. Acetic acid is the main and most abundant of these compounds. However, the production of acids such as gluconic, citric, glucuronic, tartaric, oxalic, pyruvic, L-lactic and malic can occur (Jayabalan et al., 2014).

The soluble solids content at the start was the same for all the samples. After the fermentation period, all the drinks showed a reduction in soluble solids and the mean values differed significantly from each other. There were reductions of 1.6 to 3.6 º Brix and the reduction was statistically significant ($p < 0.05$) for all the drinks.

The fermented guavira drink had the highest soluble solids content and the lowest variation in relation to the initial content. Seriguela kombucha, on the other hand, had the lowest soluble solids content and the greatest variation. This can be explained by the high amount of glucose (directly fermentable sugar) present in the seriguela pulp (Silva et al., 2016). Similarly, the higher result found in guavira kombucha may be related to the high amount of dietary fiber present in the fruit, approximately 19 g 100 g^{-1}, which could be read as soluble solids and influence the ºBrix (Novello et al., 2019).

Black and red goji berry kombuchas showed 5.80 ± 0.00 ºBrix on day 0 and 5.50 ± 0.00 ºBrix (black goji berry) and 5.60 ± 0.00 ºBrix (red goji berry) on day 11. The reduction in soluble solids content is due to the metabolization of sucrose by yeasts, transforming it into glucose and fructose and producing ethanol molecules, which serve as a substrate for bacteria to produce acetic acid (Tamer & Abuduaibifu, 2019).

The fermentation process involves the production of ethanol, which is why kombucha can have varying mLevels of alcohol. According to the Ministry of Agriculture, Livestock and Supply (MAPA), alcoholic kombucha that is safe for consumption has an alcohol content of between 0.6 and 8.0 (% v/v). However, even if kombucha is not considered alcoholic, MAPA stipulates that it is mandatory to report the presence of alcohol above 0.05 (% v/v) on labels (Brasil, 2019a).

The fermented beverages produced had significantly different alcohol content. According to current legislation, seriguela kombucha, which had the highest alcohol content (0.62 ± 0.00 % v/v), is considered an alcoholic kombucha. On the other hand, green tea kombucha (0.08 ± 0.00 % v/v) and guavira kombucha (0.26 ± 0.00 % v/v), even though they are non-alcoholic, should inform about the presence of alcohol in their composition on a possible label.

Table 3 shows the results of the microbiological analyses obtained for the three types of kombucha made.

Table 3. Microbiological count of green tea, seriguela and guavira kombuchas.

	K-Green tea	K-Seriguela	K-Guavira
Salmonella sp. (in 25 mL)	Absent	Absent	Absent
Thermotolerant coliforms (CFU mL^{-1})	< 10	< 10	< 10
Lactic acid bacteria (CFU mL^{-1})	10^7	10^7	10^6
Yeasts (CFU mL^{-1})	10^6	10^7	10^6
Acetic bacteria (CFU mL^{-1})	10^4	10^5	10^4
Total mesophilic bacteria (CFU mL^{-1})	10^5	10^4	10^5

K-Chá verde = green tea kombucha; K-Seriguela = seriguela kombucha; K-guavira = guavira kombucha.

Current Brazilian legislation does not set out a specific microbiological standard for kombucha. However, IN No. 60/2019 establishes that *in natura* beverages must be free of *Salmonella* in 25 mL of beverage and have a maximum *Escherichia coli* count of 10 CFU mL^{-1} (Brasil, 2019b). Following this standard, the three kombucha formulations prepared are within the legal standards.

The counts of lactic acid bacteria ranged from 10^6 to 10^7 CFU mL^{-1}, with the guavira kombucha sample showing the lowest count. Lactic acid bacteria can favor the growth of acetic bacteria (Ayed et al., 2016). Jayabalan et al. (2014) studied kombucha samples with a predominance of microorganisms from the *Lactobacillus* and *Gluconoacetobacter* genera. In the same study, it was found that microorganisms of the genus *Acetobacter* were less common.

Cardoso et al. (2020) counted lactic acid bacteria in green tea kombucha and obtained 10^6 CFU mL^{-1}. Despite the significant amount, only 0.015 g mL^{-1} of lactic acid was found in the final drink and acetic acid was the predominant organic acid in its composition. Seriguela kombucha had the highest yeast count, 10^7 CFU mL^{-1}. Tan et al. (2020) studied the characteristics of soursop kombucha during different fermentation periods and conditions and obtained a yeast count of 10^5 CFU mL^{-1} for a 14-day fermentation. The same amount of yeasts was also reported in a study with coffee fruit kombucha (Essawet et al., 2015). The main yeast genera found in kombucha are: *Brettanomyces/Dekkera*, *Mycoderma*, *Saccharomyces*, *Mycotorula*, *Candida*, *Mycoderma*, *Pichia*, *Koleckera*, *Schizosaccharomyces*, *Torulospora*, and *Zygosaccharomyces* (Coelho et al., 2020).

The amounts of acetic bacteria varied between 10^4 and 10^5 CFU mL^{-1} and the seriguela kombucha had the highest count. In all formulations, the amount of yeast obtained was greater than the amount of acetic acid bacteria. The same occurred when Ayed & Hamdi (2015) analyzed cactus fruit kombucha (*Opuntia ficus-indica*). One possible explanation for this is the tendency of yeasts to grow during the fermentation period. This tendency causes them to reach their maximum growth between the sixth and eighth day and then stabilize. Similarly, the amount of acetic bacteria tends to increase at the start of fermentation until it reaches its maximum growth. However, once this is reached, there may or may not be a reduction. The occurrence of a reduction in acetic bacteria may also be related to the reduction in pH during fermentation, as a very acidic pH can inhibit their growth and multiplication (De Filippis et al., 2018).

For mesophilic bacteria, the values were 10^4 to 10^5 CFU mL^{-1}. Seriguela kombucha had the lowest amount observed, possibly due to the acidity of this drink. Green tea kombucha samples fermented for 14 days had counts of 10^5 CFU mL^{-1} (Tan et al., 2020).

Mesophilic bacteria are commonly used as food hygiene indicators. High quantities of these microorganisms can indicate a lack of hygiene during the preparation and storage of the product and lead to problems such as food poisoning and food spoilage. However, quantitative analyses are not capable of differentiating between existing bacteria or identifying whether their origin is pathological. There are no established standards for the amount of mesophilic bacteria in kombucha, but it is known that fermented products have higher counts, as the microorganisms may come from the symbiotic culture (Silva et al., 2020).

Table 4 shows the values obtained in the quantification of total phenolic compounds and antioxidant activity using the ABTS and DPPH methods.

Table 4. Content of total activity of phenolic compound and antioxidant using the DPPH (EC_{50}) and ABTS (TEAC) methods for green tea, seriguela and guavira kombuchas.

	K-Green tea	K-Seriguela	K-Guavira
Total phenolic compounds (mg of GAE per mL of extract)	$1.7 \cdot 10^{3\,a} \pm 3.8 \cdot 10^{-2}$	$6.0 \cdot 10^{2\,c} \pm 2.2 \cdot 10^{-2}$	$1.2 \cdot 10^{3\,b} \pm 2.8 \cdot 10^{-2}$
EC_{50} (mL of extract per g of DPPH)	$1.4 \cdot 10^{-2\,b} \pm 10^{-3}$	$1.8 \cdot 10^{-2\,a} \pm 10^{-3}$	$1.1 \cdot 10^{-2\,c} \pm 0$
TEAC (μM of trolox per mL of extract)	$4.7 \cdot 10^{6\,ab} \pm 3.3 \cdot 10^{-2}$	$4.0 \cdot 10^{6\,b} \pm 4.0 \cdot 10^{-2}$	$6.1 \cdot 10^{6\,a} \pm 4.5 \cdot 10^{-2}$

Different lowercase letters on the same line indicate a significant difference in Tukey's test ($p < 0,05$); GAE: Gallic acid equivalent; EC_{50}: amount needed to reduce the initial DPPH radical by 50%; TEAC: Total antioxidant capacity; K-Green tea = green tea kombucha; K-Seriguela = seriguela kombucha; K-guavira = guavira kombucha.

Green tea kombucha had the highest amount of total phenolic compounds ($1.7 \cdot 10^3 \pm 3.8 \cdot 10^{-2}$ g of GAE per mL of extract), while seriguela kombucha had the lowest amount (6,0·102 ± 2.2·10-2 mg of GAE per mL of extract). All the formulations differed significantly from each other. Leonarski et al. (2021) obtained a phenolic compound content of 1309.0 mg of GAE per mL of extract for kombucha with the addition of acerola residue, fermented for 15 days, which was close to the values obtained for guavira and green tea kombuchas.

Rufino et al. (2010) carried out a study on the bioactive compounds and antioxidant activity of various fruits, classifying them in terms of phenolic compound content as: low content (< 100 mg GAE per 100 g), medium content (100 – 500 mg GAE per 100 g) and high content (> 500 mg GAE per 100 g). Alves et al., (2013) found a content of 1222.5 mg of GAE per 100g for guavira pulp, which can be considered a fruit with a high content of phenolic compounds, which may have directly influenced the content of the drink.

Looking at the EC_{50} index obtained using the DPPH method, the guavira kombucha ($1.1 \cdot 10^{-2} \pm 0$ mL of extract per g of DPPH) had the highest antioxidant potential. This is because the lower the index, the lower the amount of sample needed to reduce the DPPH radical, i.e. the higher the antioxidant potential. Seriguela kombucha ($1.8 \cdot 10^{-2} \pm 10^{-3}$ mL of extract per g of DPPH) had the highest index and therefore the lowest antioxidant potential. All the formulations differed significantly from each other. Essawet et al. (2015) obtained EC_{50} values of 0.026 to 0.170 mL of extract per g of DPPH for kombuchas added with coffee fruit at different fermentation times.

Guavira kombucha also showed the highest total antioxidant capacity (TEAC) using the ABTS method ($6.1 \cdot 10^{6} \pm 4.5 \cdot 10^{-2}$ µM of trolox per mL of extract). Similarly, seriguela kombucha ($4.0 \cdot 10^{6} \pm 4.0 \cdot 10^{-2}$ µM trolox per mL extract) had the lowest antioxidant capacity. Green tea kombucha ($4.7 \cdot 10^{6} \pm 3.3 \cdot 10^{-2}$ µM trolox per mL extract) did not differ significantly from guavira and seriguela kombuchas. However, the guavira and seriguela kombuchas differed significantly from each other. Mizuta et al. (2020) obtained TEAC values of 11,508.42 µM trolox per mL extract for green tea kombucha fermented for 14 days.

Looking at the two methods to determine antioxidant activity (DPPH and ABTS), it can be seen that the values are consistent and the formulations with the highest and lowest antioxidant capacity are the same in both methods. Rufino et al. (2010) state that the content of phenolic compounds and the antioxidant activity of a product are related, so that the higher the content of phenolic compounds, the greater the tendency for the product to have antioxidant activity. This statement is confirmed when we see that the sample with the highest content of phenolic compounds (green tea kombucha) has high antioxidant activity ($1.4 \cdot 10^{-2} \pm 10^{-3}$ mL of extract per g of DPPH and $4.7 \cdot 10^{6} \pm 3.3 \cdot 10^{-2}$ µM of trolox per mL of extract), without differing significantly from the sample with the highest TEAC (K-Guavira).

Furthermore, the addition of guavira pulp to kombucha showed a high content of phenolic compounds and an increase in the antioxidant activity of the drink, which makes the addition of this Cerrado fruit to kombucha more interesting, due to the possible beneficial properties of its consumption

Conclusion

The results obtained show that the kombuchas developed in this study meet the parameters established by current Brazilian legislation in terms of microbiological standards and standards of identity, pH and alcohol content. The guavira kombucha had volatile acidity within the legal standards. The counts of yeasts and lactic acid bacteria were higher than those of acetic bacteria in all the formulations.

As for antioxidant activity, guavira kombucha had the highest antioxidant capacity, followed by green tea kombucha and seriguela kombucha, both in the DPPH method and the ABTS method. The addition of guavira increased the antioxidant activity of the drink when compared to traditional kombucha.

Cerrado fruit kombuchas have great potential for producing bioactive compounds and beneficial properties, highlighting the importance of studies that use innovative raw materials in food preparation and that can contribute by adding nutritional, functional and sensory characteristics.

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