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Use of Kombucha SCOBY and Commercial Yeast as Inoculum for the Elaboration of Novel Beer

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Abstract: Kombucha is a beverage obtained from fermentation of *Camellia sinensis* tea using a symbiotic culture of bacteria and yeast (SCOBY). This association of bacteria and yeasts can be an interesting source of microorganisms for developing fermented beverages, including beer. The objective of this study was to evaluate kombucha SCOBY and commercial brewing yeast as a starter culture for the elaboration of beer. Three assays were performed to develop the beverage (C = control, KL = kombucha + yeast, K = kombucha). The pH, density, carbohydrates, organic acids and ethanol were evaluated during fermentation. Microbial counts (yeasts and mesophilic bacteria) and volatile compounds were recorded at the initial and final fermentation times. The content of total phenolic compounds, antioxidant capacity, color and bitterness (IBU) of the beers were determined. The results showed that kombucha-fermented wort produces a beer with differentiated characteristics. Increased lactic acid (0.73 g/L) and low alcohol content (1.3%) were observed in the K assay. Further, desired volatile compounds, such as ethyl octanoate, phenethyl acetate and 2-phenylethanol, were also found in this beer. The combination of kombucha and commercial yeast for beer production showed carbohydrate consumption and contents of organic acids similar to those of control beer, producing beers with an alcohol content of 5.9%. From the results, it was possible to observe a tendency for the content of total phenolic compounds (37.57, 33.00 and 31.64 mg/100 mL for K, KY and C assays, respectively) to increase when the wort was inoculated with kombucha. There was no difference in the antioxidant activity of the produced beers. All produced beers showed a yellowish color and a bitterness value (IBU) of 27%. The present study showed that adding kombucha as a starter culture produced beer with differentiated properties, such as high antioxidant activity, low alcohol content and sour characteristics.

Keywords: craft beer; antioxidant; kombucha; SCOBY; starter culture

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1. Introduction

Kombucha is a beverage produced from fermentation of *Camellia sinensis* infusion and sugar (sucrose) using a symbiotic culture of microbiologically active bacteria and yeasts called SCOBY, an acronym for symbiotic culture of bacteria and yeast [1]. Successful fermentation of kombucha generates a beverage containing polyphenols, ethanol, proteins and organic acids, including acetic, gluconic, glucuronic, lactic, citric, tartaric, malic and succinic acids, in addition to vitamins, amino acids and various micronutrients [2].

According to Greenwalt, Steinkraus and Ledford [3], the predominant microbial group for kombucha is acetic acid bacteria (AAB), producing acetic and gluconic acids as primary metabolites. *Gluconacetobacter xylinus* (*Acetobacter xylinum*) is the most described species

found in SCOBY and liquid kombucha. Regarding yeasts, a great diversity of species have been reported, including species of *Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Brettanomyces/Dekkera*, *Candida*, *Torula*, *Torulaspora*, *Koleckera*, *Pichia*, *Mycotorula* and *Mycoderma* [3–5].

Kombucha is a spontaneous fermentation containing great microbial diversity that can be an interesting source of microorganisms with technological interest for use in fermented beverages, including beer. Several studies have investigated the fermentation of various substrates, including grape, coffee, coconut water and others, using kombucha as inoculum with the aim of obtaining different products with distinct sensorial parameters and bioactive characteristics, such as increased phenolic compounds, anthocyanin content and antioxidant activity [6–10].

Beer is a well-known and highly appreciated product composed of malted cereals (barley or others), water, hops and yeast [11]. The combination of different ingredients leads to a great diversity of beer styles with variable alcohol content (0.05 to 54% (*v*/*v*)) [12]. Fermentation of ale and lager beers is usually undertaken using *Saccharomyces* yeasts due to their possession of essential characteristics for the fermentation process, such as ethanol production [13,14]. However, the increased consumption of beers without alcohol (<0.5% *v*/*v*) or with low alcohol content (0.5–2.0% *v*/*v*) and the search for novel beer styles has encouraged the use of non-*Saccharomyces* yeasts for beer production. The species *Dekkera/Brettanomyces* spp., *Wickerhamomyces anomalus*, *Torulaspora delbruecki*, *Lachancea thermotolerans*, *Schizosaccharomycces pombe* and *Lanchancea thermotolerans* are some examples of non-*Saccharomyces* yeasts that have been evaluated in beer fermentations [13,15].

Non-*Saccharomyces* yeasts are crucial agents for the production of sour beers. The sour character of such beers originates from a wide range of organic acids, such as lactic acid and acetic acid, produced by lactic acid bacteria, acetic acid bacteria and/or various yeasts, including *Brettanomyces* spp. [16]. Lambic beer of Belgian origin is a sour beer fermented from a consortium of bacteria and yeasts, including species belonging to the *Klebsiella*, *Enterobacter*, *Escherichia*, *Citrobacter*, *Serratia*, *Pediococcus*, *Saccharomyces* and *Dekkera* genera [13].

As kombucha has been associated with health benefits and diverse microorganisms (bacteria and yeasts) are found in the beverage, this work aimed to evaluate the chemical and antioxidant parameters of beers elaborated with kombucha SCOBY as inoculum in the presence and absence of commercial yeast beer.

2. Material and Methods

2.1. Kombucha Preparation

The SCOBY was obtained from the Laboratory of Microbial Physiology at the Federal University of Lavras (Lavras, Brazil). The kombucha was prepared with minor adaptations following a previous study [17]. First, two liters of water were boiled, and 50 g of sucrose was added. The mixture was placed in a sterilized bottle and 2 g of *Camellia sinensis* (purchased at the local market) was added, and the mixture was infused for 10 min. Then, the leaves were separated from the liquid, and after the infusion had cooled, 100 mL of kombucha liquid and SCOBY previously fermented for 10 days was added. Finally, the flask was covered with a fine cloth and incubated at room temperature (±26 °C) for 15 days.

2.2. Inoculum Preparation

The commercial yeast *Saccharomyces cerevisiae* Fermentis SafAle US-05 and kombucha (fermented tea and SCOBY) were used for wort fermentation, as described in Table 1. The yeast pitching rate was calculated using a mathematical formula from the literature [18]. A total of 2.5 g of dry commercial yeast was added to 250 mL of sterile distilled water, obtaining a final yeast population of 1.64×10^9 cells/mL. A total of 12 g of SCOBY and 10 mL of kombucha were used for inoculation in 2 L of wort during fermentation. The amount of kombucha (fermented tea and SCOBY) was calculated based on the total yeast population obtained from the SCOBY and fermented tea. The yeast populations from

the SCOBY and liquid kombucha were previously obtained using serial dilutions and plated on YEPG agar (2% *w*/*v* peptone, 1% *w*/*v* yeast extract, 2% *w*/*v* glucose, 2% *w*/*v* agar). The yeast populations found in the inoculum of the SCOBY and fermented tea were 1.45×10^9 CFU/g and 2.33×10^9 CFU/mL, respectively.

Table 1. Fermentation assays for the beer's production.

Assays	Commercial Yeast (mL)	Kombucha (mL)	SCOBY (g)
Control (C)	16.40	0	0
Kombucha + yeast (KY)	8.20	5	6
Kombucha (K)	0	10	12

2.3. Wort Production and Fermentation

Production was carried out with two replications under identical production conditions. First, the mashing of the malt was carried out using 2 kg of Pilsen Agraria-National malt (Guarapuava, Paraná, Brazil) and 15 L of filtered water. The water was heated to 60 °C, and malt was added; the mixture was kept at 65 °C for 60 min and then heated at 78 °C for 10 min. After this step, the wort was filtered, recirculated and boiled for 30 min. Then, 15 g of cascade hop pellets (6.6% iso-α-acids) was added and the mixture was boiled for an additional 30 min. The whirlpool was created, and the wort was cooled to 20 °C using an aluminum chiller. Then, 2 L of the wort was placed into 2.5 L fermenting flasks equipped with an airlock, inoculated with the yeast and kombucha as indicated in Table 1 and incubated at 20 °C for 264 h (similar conditions as for the elaboration of lager beer).

Analyses of the pH and the density of the media were performed at 0, 48, 96, 168, 216 and 264 h. The pH was evaluated using a pHmetro (Mpa-210-MS, Tecnopon, Piracicaba, Brazil), and the relative density was recorded using a floating hydrometer in the range from 1000 to 1100. After 264 h of fermentation, the beers were placed in a cold chamber (±7 °C) for ten-day maturation. Then, the beers were bottled in 600 mL bottles, and 4 g of sugar was added for carbonation. The beers were produced in duplicate and the process was repeated twice.

2.4. Analysis during Fermentation and Produced Beers

2.4.1. Enumeration of Yeasts and Bacteria

The viable counts of yeasts and mesophilic bacteria were evaluated at the initial (0 h) and final (264 h) fermentation times. Enumeration was performed using YEPG medium (2% *w*/*v* peptone, 1% *w*/*v* yeast extract, 2% *w*/*v* glucose and 2% *w*/*v* agar added with 0.01% chloramphenicol) for yeast and plate-counting agar (PCA, Merck, Rahway, NJ, USA) for bacteria [19]. The plates were incubated at 30 °C for 48 h, and the colonies were counted. Analyses were performed in duplicate.

2.4.2. Analysis of Carbohydrates, Organic Acids and Ethanol

The analyses of carbohydrates (glucose, fructose, maltose, maltotriose), organic acids (acetic acid, malic acid, lactic acid, citric acid and succinic acid) and alcohol (ethanol) were performed during fermentation in accordance with previous studies [20]. A Shimadzu liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with a dual detection system consisting of a UV–visible detector (SPD 10Ai) (for acids) and a refractive index detector (RID-10Ai) (for carbohydrates and alcohol) was used. The samples were centrifuged at room temperature (23–26 °C, 14,000× *g*) for 5 min, filtered through a 0.45 UM/30 mm membrane filter and transferred to 2 mL microtubes, and then 10 µL was used for HPLC analysis. Analyses were performed in triplicate. Carbohydrate, alcohol and organic acid analyses were performed using a Shimadzu SCR 101-C ion exclusion column (7.9 mm × 300 mm i.d., 10 µm) at a flow rate of 0.5 mL/min, operating at 80 °C and using ultrapure water as the mobile phase for carbohydrates and alcohols and operating at 50 °C and using aqueous perchloric acid solution as the mobile phase for organic acids.

The concentration of compounds was measured by comparing the corresponding peak area with the standard curve and multiplying by the dilution factors. All samples were analyzed in triplicate.

2.4.3. Analysis of Volatile Compounds by GC-MS

The volatile compounds of the samples at 0 h, at the end of fermentation and after ten days of maturation were analyzed. The extraction and analysis parameters were set as described by Zhang et al. [21] with minor modifications, using headspace manual solid phase microextraction (HS-SPME) with a 2 cm DVB/CAR/PDMS 50/30 µm SPME fiber (Supelco, Bellefonte, PA, USA). An aliquot of 3.0 mL of samples was added to a 20 mL vial, equilibrated at 60 °C for 15 min and then exposed to sample vials for 30 min at the same temperature. The analyses were performed using a GC–MSQP2010.

An SE system (Shimadzu) equipped with a DB-WAX column (30 m × 0.25 mm i.d. × 0.25 µm) was used at 50 °C for 5 min; then, the temperature was increased at a rate of 3 °C/min up to 190 °C and maintained at 190 °C for 10 min. High-purity helium was used as a carrier gas, with a constant flow of 1.2 mL/min. The injections were performed in splitless mode with an injection time of 2 min. The ion source temperature was 230 °C, and the quadrupole's temperature was 150 °C. The detected mass spectra were compared with the NIST11/NIST11a database, and an alkane series (C10–C40) was used to calculate the retention index (RI). Analyses were performed in duplicate.

2.4.4. Total Phenolics

The determination of the total phenolics in the beers obtained was carried out according to a methodology previously described [22]. Samples of 0.5 mL of each beer were added to the test tube. Then, 3 mL of distilled water and 0.250 mL of Folin Ciocalteau reagent were added. The solution was homogenized, and 1 mL of saturated sodium carbonate solution (Na_2CO_3) was added. After 30 min of incubation at room temperature (23–26 °C), the absorbance was evaluated in triplicate at 750 nm in a digital UV–Vis spectrophotometer (ESPEC-UV-5100 Tecnal, Piracicaba, Brazil). Results were expressed in gallic acid equivalents.

2.4.5. Antioxidant Capacity Determined by the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Test

The DPPH radical scavenging test was performed as previously described [23] with minor modifications to evaluate the antioxidant capacity of the beers. Samples of 100 µL of beer were added to 1.9 mL of the DPPH radical solution diluted in methanol (1:1) and stored in the dark for 30 min at room temperature (23–26 °C). The ability to scavenge free radicals was evaluated by measuring the absorbance at 517 nm. Results were presented as the means of inhibition percentages. Analyses were performed in triplicate.

2.4.6. Color Analysis

The color analysis of the beers was performed using a Konica Minolta CR5 bench colorimeter (Tokyo, Japan). It was undertaken through L*, a* and b* measurements with the Standard Reference Method (SRM).

2.4.7. Bitterness Analysis

The bitterness of the beers was determined in International Bitterness Units (IBUs) using the standard formula: IBU = U × P × A/V (2), where U is the hop utilization; P is the hop weight (mg); A is the α-acid hop units (in decimals); and V is the volume of beer used.

2.5. Statistical Analysis

The results were expressed as means ± standard deviation (SD). The one-way analysis of variance (ANOVA) test was used to analyze the data and the Tukey test to compare the

differences between the means with a significance level of $p < 0.05$, while Past 4 software, Excel and GraphPad Prism 8 were used for graphics assembly.

3. Results and Discussion

3.1. pH and Density

The initial pH values of the assays were 5.51, 5.46 and 5.46 for the C, KY and K assays, respectively. There was a slight decrease in pH values during fermentation, with the final values remaining at 4.37, 4.63 and 4.15 for the C, KY and K assays, respectively. These values are generally found in beers. The decrease in pH occurs due to the production of carbonic acid from carbon dioxide (CO_2), the production of organic acids and the consumption of buffering compounds (basic amino acids and primary phosphates) in the wort during fermentation [24].

Regarding density values, it was possible to observe a decrease in the values. The initial value of the wort was 1055.5. At the end of the fermentation, the C and KY assays presented values of 1015 and 1013, respectively, while the K assay presented a density of 1038.5. The C and KY values were consistent with those found in the literature representing the density values of conventional beers [25]. However, the final value of K differed from the values for the density of commercial beers and special styles [26]. This was because the C and KY assays were inoculated with the commercial yeast *S. cerevisiae*, while K was only inoculated with the kombucha culture. This fact may explain the differences in the final density values, since the commercial yeast efficiently consumed the fermentable sugars of the wort, as evidenced by the result for the carbohydrate consumption (Figure 1).

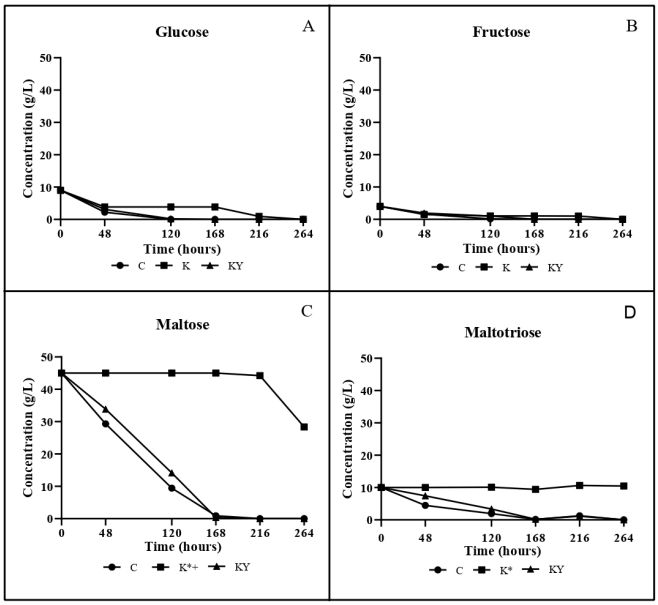

Figure 1. Concentrations (g/L) of glucose (**A**), fructose (**B**), maltose (**C**) and maltotriose (**D**) during the 264 h of fermentation for the C, K and KY assays. C: control, K: kombucha, KY: kombucha + commercial brewer yeast. * Significant difference ($p < 0.05$) between C and K assays; +, significant difference ($p < 0.05$) between K and KY assays.

The density is related to the amount of fermentable carbohydrates available and their consumption in the wort. The reductions in the carbohydrate concentration and the density during fermentation are directly related to the production of alcohol in beer [27]. In the present study, we found that the K assay, containing only kombucha as inoculum, showed a lower value for the alcohol content (1.3%) than the assays containing the commercial yeast *S. cerevisiae* (5.9% for the C and KY assays). These values follow the alcohol content parameters for beers [12]. However, the K assay resulted in a beer with low alcohol content.

3.2. Microbial Population

The microbial counts performed at the initial and final fermentation times are shown in Table 2. An increase in the viable counts of yeast was observed in all fermentations. Regarding the viable counts of mesophilic bacteria, there was an increase in the assay inoculated only with kombucha. However, in the assay inoculated with kombucha and yeast (KY), the bacterial population remained constant, which may have been due to competition between the bacteria from the kombucha and commercial yeast. As expected, no bacteria were detected in the control assay, as it was inoculated only with commercial yeast. Kombucha contains many microorganisms, including bacteria and yeasts, which may grow in different substrates [6–10]. Although the carbohydrates present in the k assay were not completely fermented by kombucha microorganisms, it was possible to observe yeast growth, probably mostly non-*Saccharomyces*, until 264 h of fermentation. In the present study, the wort was found to be an adequate substrate for kombucha microorganisms, especially yeasts, since this group was able to grow during all fermentation assays.

Table 2. Viable counts of yeast and mesophilic bacteria evaluated at the initial and final fermentations times in the three different assays.

	Population (CFU/mL)	
Yeast	**Initial Time**	**Final Time**
Control (C)	$1.12 \times 10^9 \pm 1.24$	$2.25 \times 10^{10} \pm 1.62$
Kombucha (K)	$7.03 \times 10^8 \pm 3.29$	$3.00 \times 10^{10} \pm 2.09$
Kombucha + yeast (KY)	$6.50 \times 10^8 \pm 2.69$	$2.00 \times 10^{10} \pm 2.77$
Bacteria		
Control (C)	ND	ND
Kombucha (K)	$7.40 \times 10^9 \pm 3.68$	$1.01 \times 10^{10} \pm 1.41$
Kombucha + yeast (KY)	$1.66 \times 10^{10} \pm 9.33$	$1.60 \times 10^{10} \pm 1.69$

ND, not detected.

3.3. Carbohydrates, Ethanol and Organic Acids Analyses

The maltose, glucose, fructose and maltotriose were analyzed. The data are shown in Figure 1. The most abundant fermentable carbohydrates in wort are maltose (50–60%), maltotriose (15–20%) and glucose (10–15%), the remainder being represented by fructose (5–10%), sucrose (3–6%) and non-fermentable sugars (20–30%) [28], which corroborates our findings. For beer production, it is crucial that the microorganisms, especially the yeasts, can metabolize the carbohydrates to produce ethanol, carbon dioxide and secondary metabolites, which may affect the sensorial characteristics of the beverage [29].

It was observed that the initial glucose concentration (around 10 g/L) was mainly consumed within the first 120 h of fermentation in the C and KY assays, reaching values of 0.04 g/L and 0.19 g/L, respectively. In the K assay, the decrease in glucose was observed after 216 h, reaching a concentration of 0.95 g/L. This fact was probably related to the absence of commercial brewer's yeast in the K assay. The genus *Saccharomyces*, among others, efficiently metabolizes wort sugars and ensures the production of ethanol and carbon dioxide from beer [24]. Regarding fructose, low concentrations ($\leq$5 g/L) were observed at the beginning of fermentation, and it was no longer detected at the end of all fermentations. It was observed that the glucose and fructose were consumed first, followed by the maltose and maltotriose. In general, the utilization of carbohydrates by yeasts occurs sequentially, with glucose being consumed more quickly; then, when approximately half of this sugar is consumed, maltose and maltotriose are metabolized [28]. In *Saccharomyces* spp. yeast, the metabolization of maltotriose is even slower than maltose. Therefore, this sugar can be found in the final product, generating an atypical flavor and low ethanol yield [30]. In the present study, glucose and fructose were almost totally consumed by 120 h, while maltose and maltotriose were consumed by 168 h for the C and KY assays. The K assay showed different behavior, probably due to the absence of commercial yeast. In the C and

KY assays, there was a decline in maltose concentration until 168 h (from around 45 g/L to lower than 1 g/L). In comparison, in the K assay, the main decline was observed at 216 to 264 h of fermentation, showing a considerable residual concentration (28 g/L). Maltotriose decreased from around 10 g/L at time 0 to 0.03 g/L at 168 h of fermentation in the C and KY assays. However, in the K assay, maltotriose remained almost constant (around 10 g/L) throughout the entire fermentation time.

In a previous study [31], *Lachancea fermentati* strain was isolated from kombucha and evaluated as a starter culture to produce beer with low alcohol content. At the end of fermentation, the authors observed the presence of residual maltotriose in the wort and total glucose consumption. In another work, non-*Saccharomyces* strains isolated from kombucha (*Zygosaccharomyces kombuchaensis*, *Hanseniaspora vineae*, *Hanseniaspora valbyensis*, *Torulaspora delbrueckii*, *Zygosaccharomyces bailii* and *Saccharomycodes ludwigii*) were employed to produce beer without alcohol. The authors noted a low ability to ferment maltose and maltotriose among these yeasts in contrast to the consumption of glucose and fructose [31]. Some non-*Saccharomyces* yeast species have shown a low capacity to ferment maltose and maltotriose [15,32]. These findings, in agreement with our results, demonstrate a potential use of kombucha inoculum to produce low-alcohol beers. In the present study, the ethanol content of the assay employing only kombucha as inoculum was 13.10 g/L.

Ethanol was produced in all assays, as observed in Figure 2. The increase in ethanol concentration followed the metabolism of carbohydrates detected in the wort for the different assays. The C and KY assays showed the highest ethanol increase from 48 h until 168 h, while for the K assay, the main increase occurred from 168 h to 264 h (Figure 2). The K assay presented lower assimilation of maltose and maltotriose than the C and KY assays; consequently, the final ethanol concentration was around four times lower (59.97, 59.39 and 13.10 g/L for C, KY and K, respectively, at the end of fermentation). It seems that the microorganisms from kombucha in the K assay needed a longer fermentation period to adapt to the wort and initiate the assimilation of carbohydrates and ethanol production.

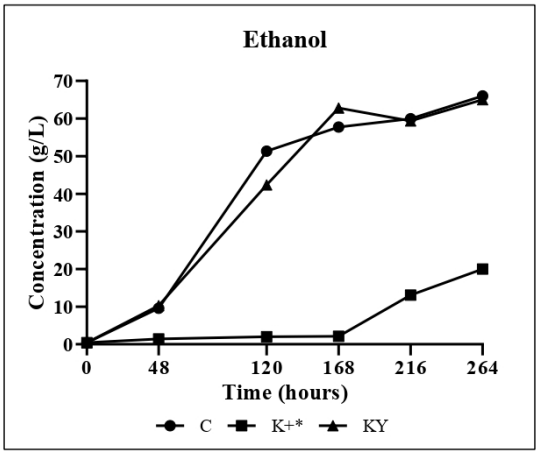

Figure 2. Ethanol production during 264 h of fermentation in the C, KY and K assays. C: Control, K: kombucha, KY: kombucha + commercial brewer yeast. * Significant difference ($p < 0.05$) between C and K assays; +, significant difference ($p < 0.05$) between K and KY assays.

Traditional kombucha fermentations using tea as a substrate generate lower ethanol concentrations than the K assay in the present study using wort as a substrate. In the K assay, 20.49 g/L of ethanol was obtained after 11 days of fermentation, while traditional kombucha fermented at 20 °C for 10 days was found to result in 10.7 g/L [24]. The authors of [15] found a lower ethanol concentration (0.28 g/L) in the fermentation at 7 days, following which a decrease (0.14 g/L) was observed after 14 days of fermentation. These findings indicate that the use of wort as a substrate, along with the fermentation conditions, may favor the metabolism of kombucha yeasts. The ethanol content of the beers was 5.9%

for the C and KY assays and 1.3% for the K assay. According to the Brazilian legislation classifications, the beers obtained from the K assay were low-alcohol beers [12].

Organic acids are important chemical components produced by microbial fermentation. In addition, they play an essential role in beer production, as they contribute to flavor, color and aroma properties and are good indicators of fermentation performance [33]. In the present study, succinic acid was detected in more significant amounts in the C and KY assays, reaching values of 2.7 and 2.47 g/L, respectively. On the other hand, lactic acid was the main acid detected in the K assay, showing a concentration of 0.73 g/L. The organic acids detected during the fermentation are illustrated in Figure 3.

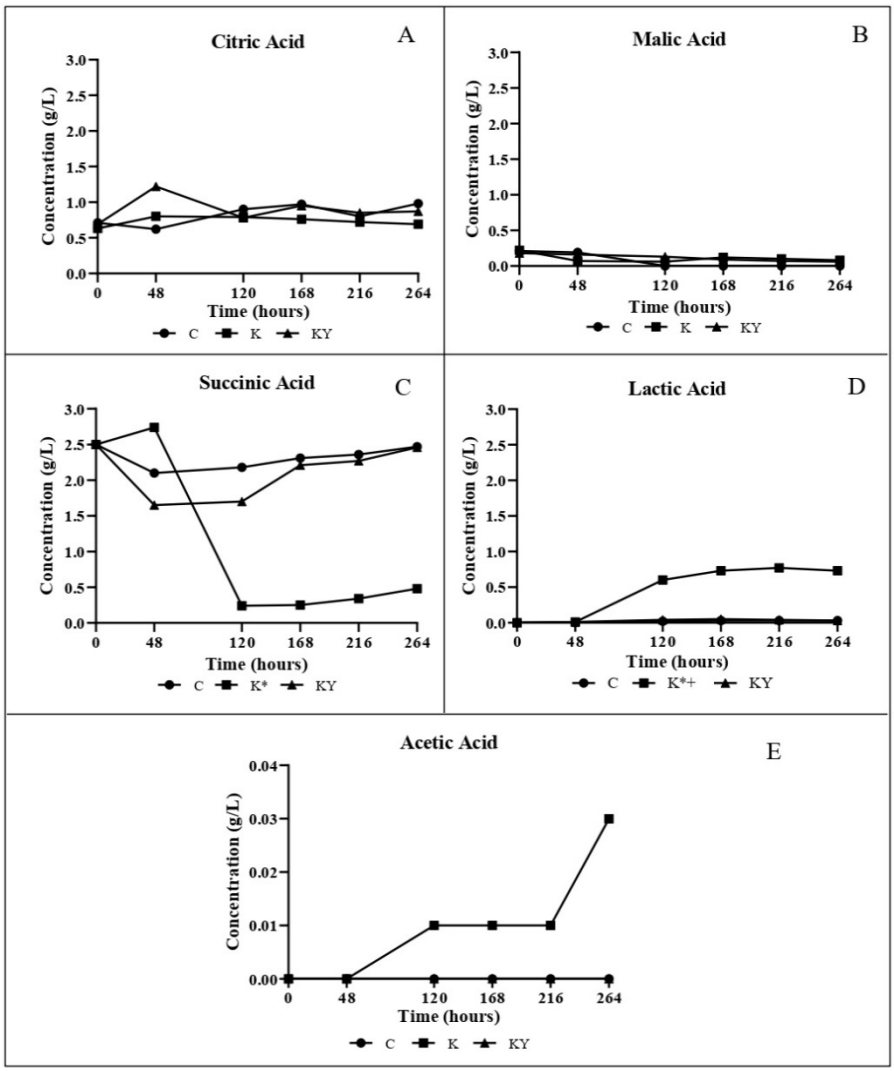

Figure 3. Evaluation of lactic acid (**A**), malic acid (**B**), succinic acid (**C**) and citric acid (**D**) during 264 h of fermentation in the C, KY and K assays. Acetic Acid (**E**). C: Control, K: kombucha, KY: kombucha + commercial brewer yeast. * Significant difference ($p < 0.05$) between C and K assays; +, significant difference ($p < 0.05$) between K and KY assays.

Lactic acid was produced only in the K assay from 48 h of fermentation and reached a concentration of 0.73 g/L at the end of fermentation. This fact may have been due to the K assay being inoculated only with kombucha (without commercial yeast), which has been described as possessing diverse microorganisms, including acetic acid bacteria, yeasts and, to a lesser extent, lactic acid bacteria [34,35]. Lactic acid is mainly produced by lactic acid bacteria, as well as by some yeasts. Although the KY assay was inoculated with kombucha, commercial yeasts were also added, which may have competed with the

kombucha microorganisms. Traditional kombucha prepared using green and black tea as substrate and fermented for 12 days at 24 °C resulted in 0.12 and 0.24 g/L, respectively, of lactic acid [2]. These values are lower than the present study's findings, which may have been due to the substrate and the fermentation conditions. In general, kombucha is aerobically fermented. However, in the present work, the anaerobic condition was stimulated by the closed flasks used for fermentation, which may have favored lactic acid bacteria growth. Malic acid showed a decrease from 0.2 g/L to around 0.01 g/L in all assays. Decreases in levels of malic acid from approximately 0.5 g/L to 0.3 g/L have been reported for beers fermented with non-*Saccharomyces* strains [32]. According to the authors, the reduction in malic acid could have been due to the cellular uptake and retention of this acid involving passive diffusion or proton symport transfer. Citric acid remained almost constant at low concentrations (approximately 0.07 g/L) during all fermentation periods in all assays. Citric acid is generally a key compound of the tricarboxylic acid cycle in cell metabolism and may be produced by brewer's yeast. This acid can contribute to the acidity of beer.

Regarding succinic acid, it was observed that the assays inoculated with kombucha (K and KY) showed more accentuated declines in this acid up to 120 h of fermentation, especially in the K assay, where it reached a value of 0.49 g/L at the end of fermentation. However, in the KY assay containing the commercial yeast, there was an increase in succinic acid from 120 h to 168 h and it reached a final value of 2.47 g/L, which may have been due to yeast metabolism. Succinic acid is produced during aerobic respiration as an intermediate in the tricarboxylic acid cycle. Furthermore, it is also one of the products of fermentation during anaerobic metabolism [36]. Succinic acid imparts a salty and bitter taste to a beverage [37]. The C assay showed constant values throughout the fermentation, varying from around 2.0 to 2.5 g/L. Organic acids, such as lactic, malic, succinic, citric and acetic acids, have been reported in kombucha, and their concentration may vary with different fermentation conditions [9,38,39].

Acetic acid was detected in low concentration in all fermentation assays, showing concentrations of 0.01 g/L for the C and KY assays and 0.04 g/L for the K assay at the end of fermentation (Figure 3). Acetic acid showed a slight increase from 168 h in the K assay (0.01 to 0.05 g/L). The presence of acetic acid bacteria in traditional tea kombucha is well-documented; consequently, acetic acid is the major organic acid detected, reaching values around 11–12 g/L [4,5]. However, using wort as a substrate and fermentation conditions such as closed flasks did not favor acetic acid production, since acetic acid bacteria are strictly aerobic microorganisms. Acetic acid confers a vinegary flavor and, in large amounts, is not desirable in most beer styles.

3.4. Analysis of Volatile Compounds by GC-MS

A total of 37 volatile compounds were identified at the initial fermentation time (0 h), final time (264 h) and after 10 days of maturation in a cold chamber (FM) (Table 3). The identified compounds were grouped into acids (11), alcohols (8), esters (12), phenols (1) and terpenes (5). Esters, acids and alcohols were the largest groups of volatile compounds detected. In general, ester compounds were not detected at the initial time of fermentation but were produced during fermentation and remained during the maturation stage. Esters are important aromatic elements of beer produced by yeasts that generally define the fruity and floral notes of the beverage [40,41]. Phenethyl acetate has a rose and honey aroma [42], and it was mainly found in the C and KY assays, showing peak areas of 97.4×10^4 and 64.0×10^4 after maturation. This compound was probably related to the commercial yeast metabolism. On the other hand, ethyl octanoate (with an apple and fruity flavor) was mainly found in the assays inoculated with kombucha (K and KY), particularly at the end of maturation (peak areas of 11.4×10^4 and 45.0×10^4 in the KY and K assays, respectively), indicating higher production by kombucha microorganisms. Furthermore, ethyl isopentyl succinate and ethyl succinate were only found in the assays inoculated with kombucha.

Table 3. Volatile compounds (peak area × 10^4) detected at 0 h and 264 h of fermentation and after 10 days of maturation for beers produced in the assays inoculated with commercial yeast (C), kombucha and commercial yeast (KY) and kombucha (K).

	C			KY			K		
	Peak Area × 10^4								
	0 h	**264 h**	**FM**	**0 h**	**264 h**	**FM**	**0 h**	**264 h**	**FM**
Acids									
Heptanoic acid	2.6	1.8	2.1	3.2	1.4	1.4	1.1	1.4	2.2
Nonanoic acid	5.0	1.8	2.1	5.4	1.9	1.1	1.5	1.7	2.5
n-Decanoic acid	3.7	38.7	15.6	3.8	13.7	11.5	0.8	29.2	37.8
9-Decenoic acid	0.9	1.4	4.1	1.6	ND	2.2	ND	0.4	5.8
Dodecanoic acid	2.3	5.6	4.5	2.3	3.2	5.0	0.3	9.6	11.3
Tetradecanoic acid	4.0	1.9	2.0	4.9	2.9	1.8	0.8	2.2	3.0
Pentadecanoic acid	0.7	0.4	0.7	0.8	0.5	0.2	ND	0.3	0.7
n-Hexadecanoic acid	49.5	12.4	12.3	45.5	19.7	7.3	6.6	9.9	16.9
Octadecanoic acid	1.7	0.7	0.6	1.7	0.7	0.6	1.0	0.8	0.6
Hexanoic acid	9.0	10.2	12.0	11.3	4.5	5.4	4.3	28.4	26.3
Octanoic acid	5.8	217.2	180.2	6.8	156.5	152.0	1.9	115.7	182.0
Alcohols									
1-Octanol	580.9	41.8	53.5	1615.3	480.0	18.2	148.3	319.1	1155.2
1-Nonanol	2.8	5.7	3.6	2.4	2.2	4.5	2.8	2.2	8.8
L-.Alpha.-terpineol	2.4	0.4	0.6	3.1	0.9	0.8	2.0	0.7	0.6
2-PhenyIethanol	17.9	875.5	1049.0	21.5	626.8	662.5	13.1	533.2	672.3
1-Dodecanol	2.6	2.7	2.9	2.5	4.9	4.4	0.6	4.2	6.0
1-Hexadecanol	ND	1.4	1.3	ND	0.5	1.4	ND	2.5	4.5
Benzyl alcohol	1.3	0.8	0.9	1.1	0.8	0.9	2.0	0.6	0.7
1-Tetradecanol	ND	2.7	4.4	ND	2.5	4.6	ND	2.4	3.2
Esters									
Ethyl succinate	ND	ND	ND	ND	ND	ND	ND	61.6	98.5
2-Phenylethyl acetate	ND	112.7	97.4	ND	93.4	64.0	ND	23.7	21.5
Ethyl decanoate	ND	263.5	36.4	ND	237.2	66.5	ND	298.9	199.4
Ethyl octadec-9-enoate	ND	1.7	1.1	ND	0.3	1.9	ND	0.9	5.5
Ethyl isopentyl succinate	ND	ND	ND	ND	ND	ND	ND	5.2	6.4
Ethyl pentadecanoate	ND	0.6	0.6	ND	0.05	1.6	ND	16.4	5.8
Ethyl hexadecanoate	2.1	88.9	40.4	3.3	19.2	79.9	1.9	46.2	65.4
Ethyl tetradecanoate	ND	24.4	11.3	ND	5.2	23.6	ND	133.2	106.4
Ethyl cinnamate	ND	0.8	0.8	ND	0.7	0.9	ND	0.6	1.4
Phenethyl isovalerate	ND	3.1	3.2	ND	ND	ND	ND	ND	ND
Ethyl octanoate	ND	7.7	6.9	ND	2.3	11.4	ND	27.3	45.0
Methyl 9,12-Octadecadienoate	1.1	10.4	6.4	1.5	2.7	14.2	1.3	52.7	61.4
Phenols									
4-Ethyl-2-methoxy-phenol	0.2	1.4	3.6	1.3	22.8	34.4	0.7	310.9	311.9
Terpenes									
Citronellol	ND	11.5	12.9	ND	12.0	13.0	ND	12.8	15.8
Geraniol	69.7	20.9	20.0	74.5	35.5	26.3	37.5	40.6	31.8
Geranyl acetate	5.6	ND	ND	4.3	ND	ND	3.6	ND	ND
Humulene	14.4	58.1	5.0	23.7	23.9	6.3	7.8	75.8	3.7
Caryophyllene oxide	5.9	ND	ND	4.6	ND	ND	2.9	ND	ND

ND, not detected.

A great variety of acids were found in all assays. Octanoic acid (fatty, oily, rancid aroma) was the main acid found and may have been produced during beer conditioning. This compound may, in high concentrations, confer an off flavor to a beer. Regarding alcohols, 1-hexadecanol and 1-tetradecanol were not detected at 0 h and were produced during the fermentation and maturation stages. 2-Phenylethanol (sweetish and floral flavors) is the desired compound in fermented beverages, and it was detected in all assays; it was mainly produced during fermentation and maturation. This compound has been associ-

ated with Saccharomyces and non-*Saccharomyces* activity (e.g., *T. delbrueckii* strains) [31,43]. Furthermore, beers produced by co-culturing of *S. cerevisiae* and lactic acid bacteria have demonstrated higher amounts of 2-phenylethanol than single cultures [44]. The authors suggest that this can be attributed to phenylalanine and leucine release via proteolysis by lactic acid bacteria.

Brettanomyces yeasts produce the phenolic compound 4-ethyl-2-methoxy-phenol (4EG) by transforming vinyl phenols catalyzed by vinyl phenol reductases [45]. Higher levels of 4EG may be desired for some beer styles—mainly those are spontaneously fermented, including lambic beers—and confer a spicy "Brett" character. 4EG was detected in all assays, especially after fermentation in the K assay, which may be attributable to the kombucha microorganisms' metabolism. Regarding terpenes, these compounds are present in hops and are responsible for beer aroma [46]. The most common terpenes found in all assays were geraniol, which gives a floral aroma (rose and geranium) to beer; citronellol (lemon, lime); and humulene. Humulene is the main terpene found in cascade hops and imparts a citrusy, floral, woody and earthy aroma to products [46,47]. Geranyl acetate and caryophyllene oxide were only detected at 0 h and were probably metabolized in all assays.

3.5. Total Phenolic Compounds and Antioxidant Activity of the Produced Beers

The total phenolic compounds in the beers were evaluated (Table 4), and there were no significant differences ($p > 0.05$) among the three assays evaluated. However, beer produced from the K assay presented a tendency ($p = 0.12$) for the total phenolic compounds content to increase (37.57, 33.00 and 31.64 mg/100 mL for K, KY and C assays, respectively). No significant difference ($p > 0.05$) was observed for antioxidant activity.

Table 4. Total phenolic compounds, DPPH analysis, color and bitterness of the produced beers.

Analysis	Control (C)	Kombucha (K)	Kombucha + Yeast (KY)
Total phenolic compounds (mg/100 mL)	31.64 ± 1.40	37.57 ± 1.59	33 ± 1.80
DPPH (% inhibition)	63.00 ± 6.46	69.04 ± 5.86	65.1 ± 4.79
L*	87.92 ± 0.21	82.56 ± 6.55	86.16 ± 1.79
a*	0.4 ± 0	1.82 ± 0.70	1.46 ± 1.03
b*	21.31 ± 1.07	23.88 ± 2.28	19.87 ± 0.08
Bitterness (IBU %)	27	27	27

The microorganisms in kombucha release enzymes during fermentation, which can break down polyphenols into small molecules. Therefore, the increased phenolic compound content in the samples containing kombucha could have been derived from phenolics, including flavonoids [48]. In another study [49], higher polyphenol content was observed in fermented kombucha compared to unfermented black tea, highlighting the importance of microbial activity in conferring functional properties to beverages. Furthermore, studies using soy whey [8] and coconut water [50] as the substrate for kombucha fermentation demonstrated a significant increase in the percentage of inhibition of the DPPH radical compared to unfermented substrates.

3.6. Color and Bitterness of the Produced Beers

The color analysis showed that the three beers had L* values close to 100 (C = 87.92 ± 0.21; K = 82.56 ± 6.55; KL = 86.16 ± 1.79), indicating a clear beer. When performing the beer color analysis with the SRM method, it was considered that the L* value could be between 0 and 100, and higher L* values corresponded to clearer samples. In addition, the a* value corresponded to a red versus green color spectrum, indicating that, with a higher a* value, the samples were redder (C = 0.4 ± 0; K = 1.82 ± 0.70; L = 1.46 ± 1.03). The b* value corresponded to a yellow versus blue color spectrum, with higher b* values corresponding to more yellowish samples [51]. From the results, it was observed that the beers had a yellowish color in addition to being clear.

Based on the formula used for the bitterness analysis, the beer's bitterness value (IBU) was 27%. The IBU percentage is related to the concentration of iso-alpha-acids, and higher rates correspond to more bitterness in beers. The hop flavor enhancement becomes noticeable from approximately 35% IBU [27]. Thus, the produced beverages may be considered low-bitterness beers.

4. Conclusions

Fermentation of wort using kombucha as a starter culture resulted in a beverage with differentiated characteristics compared to the control beer using only commercial yeast. The beer inoculated with kombucha showed increased lactic acid and low alcohol content. Further, desired volatile compounds, such as ethyl octanoate, phenethyl acetate and 2-phenylethanol, were also found. The combination of kombucha and commercial yeast for beer production showed carbohydrate consumption and contents of organic acids and alcohol similar to those of the control beer. Regarding the functional properties of the beverages, it was possible to observe a tendency for the total phenolic compound content to increase when the wort was inoculated with kombucha. The present study showed that it is possible to produce beer with differentiated properties, such as low alcohol content and sour characteristics, by adding kombucha as a starter culture. These findings are an important step toward developing low-alcohol beers with differentiated characteristics. However, more studies are crucial, including microbial identification and optimization of fermentation parameters (e.g., time and temperature), to obtain better knowledge and standardization of the process for industrial applications.

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