

Physicochemical, antioxidant and sensory properties of Kombucha beverages obtained from oolong or yerba mate tea fermentation

Propriedades físico-químicas, antioxidantes e sensoriais de Kombuchas obtidas da fermentação de oolong e erva mate

Propiedades fisicoquímicas, antioxidantes y sensoriales de las Kombuchas obtenidas de la fermentación de oolong y yerba mate

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Abstract

Kombucha is a non-alcoholic fermented beverage traditionally produced from a sugared tea that presents a sour and refreshing taste. This work aimed to develop, characterize and evaluate the sensory acceptance of Kombucha beverages made from oolong, a traditionally tea used for Kombucha production, or yerba mate tea, which is very popular and easily found herb in Brazil. The characterization was related total soluble solids (TSS), sugars, pH, titratable acidity (TA), organic acids (OA), alcohol content (AC), phenolics (PHE), flavonoids (FL), antioxidant activity (AA), besides, the sensory acceptance by potential consumers. Total soluble solids decreased 8.3% in formulation with oolong and 7.0% in formulation with yerba mate tea. The pH ranged from 4.3 and 4.5 to 2.8 and 3.1 after 14 days, respectively. Titratable acidity reached 8.97 g.L⁻¹ in oolong tea and 6.75 g.L⁻¹ in yerba mate tea. Acetic acid was the highest organic acid identified and quantified at the end of fermentation time. Flavonoids decreased during fermentation in both samples, while reducing capacity did not differ throughout fermentation time. Regarding the antioxidant capacity, in the formulations with oolong and yerba mate, it presented higher inhibitory capacity of the ABTS radical of 90.22 and 68.75%, while the DPPH radical inhibitory capacity was 89.74% in oolong and 86.72% in yerba mate. Kombucha formulated with yerba mate tea resulted in a sour and refreshing beverage, with higher global acceptance compared to oolong tea, both drinks exhibiting antioxidant potential *in vitro*.

Keywords: Fermentation; Non-alcoholic beverage; Reducing capacity; Sensory acceptance.

Resumo

O Kombucha é uma bebida fermentada não alcoólica, tradicionalmente produzida a partir de um chá açucarado, apresenta um sabor ácido e refrescante. Este trabalho teve como objetivo, desenvolver, caracterizar e avaliar a aceitação sensorial de Kombucha à base de oolong, tradicionalmente utilizado para a produção desta bebida e erva-mate, muito popular e facilmente encontrada no Brasil. Os produtos foram caracterizados quanto aos sólidos solúveis totais (SST), açúcares, pH, acidez titulável (AT), ácidos orgânicos (OA), teor de álcool (AC), fenólicos (PHE), flavonóides (FL), atividade antioxidante (AA), além da aceitação sensorial por potenciais consumidores da bebida. Os

SST diminuíram 8,3% na formulação com oolong e 7,0% na formulação com chá de erva-mate. O pH variou de 4,3 e 4,5 para 2,8 e 3,1 após 14 dias, respectivamente. A acidez titulável atingiu 8,97 g.L⁻¹ no Kombucha oolong e 6,75 g.L⁻¹ no de erva-mate. O ácido acético foi o maior ácido orgânico identificado e quantificado ao final do tempo de fermentação. Os flavonóides diminuíram durante a fermentação em ambas as amostras, enquanto a capacidade redutora não diferiu neste processo. Em relação à capacidade antioxidante, nas formulações com oolong e erva mate apresentou maior capacidade inibitória do radical ABTS de 90, 22 e 68,75%, enquanto a capacidade inibitória do radical DPPH foi de 89,74 para oolong e 86,72% para erva-mate. O Kombucha formulado com chá de erva-mate resultou em uma bebida ácida e refrescante, com maior aceitação global em comparação ao chá oolong, ambas as bebidas exibiram potencial antioxidante *in vitro*.

Palavras-chave: Fermentação; Bebida não alcoólica; Reduzindo a capacidade; Aceitação sensorial.

Resumen

La kombucha es una bebida fermentada sin alcohol, tradicionalmente producida a partir de un té azucarado, tiene un sabor ácido y refrescante. Este trabajo tuvo como objetivo desarrollar, caracterizar y evaluar la aceptación sensorial de Kombucha a base de oolong, tradicionalmente utilizado para la producción de esta bebida y mate, muy popular y fácil de encontrar en Brasil. Los productos se caracterizaron por sólidos solubles totales (TSS), azúcares, pH, acidez titulable (TA), ácidos orgánicos (OA), contenido alcohólico (AC), fenólicos (PHE), flavonoides (FL), actividad antioxidante (AA), además de la aceptación sensorial por parte de los bebedores potenciales. El TSS disminuyó un 8,3% en la formulación de oolong y un 7,0% en la formulación de té de yerba mate. El pH osciló entre 4,3 y 4,5 a 2,8 y 3,1 después de 14 días, respectivamente. La acidez titulable alcanzó 8,97 g.L⁻¹ para Kombucha oolong y 6,75 g.L⁻¹ para yerba mate. El ácido acético fue el ácido orgánico más grande identificado y cuantificado al final del tiempo de fermentación. Los flavonoides disminuyeron durante la fermentación en ambas muestras, mientras que la capacidad reductora no difirió en este proceso. En relación a la capacidad antioxidante, en las formulaciones con oolong y yerba mate presentó mayor capacidad inhibitoria del radical ABTS de 90.22 y 68.75%, mientras que la capacidad inhibitoria del radical DPPH fue de 89.74 para oolong y 86.72% para yerba mate. La kombucha formulada con té de yerba mate resultó en una bebida ácida y refrescante, con mayor aceptación global en comparación con el té oolong, ambas bebidas exhibieron potencial antioxidante *in vitro*.

Palabras clave: Fermentación; Bebida sin alcohol; Reducción de capacidad; Aceptación sensorial.

1. Introduction

Kombucha is a traditional Asian beverage, also called "tea fungus", which is obtained from the fermentation of sugared tea by the association between bacteria and yeasts (Kallel et al. 2012; Watanawa et al. 2015a; Gomes et al. 2018; Dantas Coelho et al. 2020; Laavanya, Shirkole & Balasubramanian 2021). Kombucha presents a refreshing and enjoyable sour taste, and also exhibited many therapeutic and functional benefits by acting as prophylactic agent on maintaining human health (Četojević-Simin et al. 2012; Jayabalan et al. 2014; Gomes et al. 2018). Although few studies involving the effects of Kombucha beverages in humans have been developed, there are reports in the literature, as *in vitro* as *in vivo* using animal model, whose effects are related to improved the digestion, relief from arthritis, acting as a laxative, preventing microbial infections, combats stress and cancer, provides relief against hemorrhoids, imparts a positive influence on the cholesterol levels, facilitates excretion of toxins and shows antioxidant activity (Watawana et al. 2015a; Dantas Coelho et al. 2020; Laavanya, Shirkole & Balasubramanian 2021). Moreover, Kombucha beverage also can contribute to consumers' health due to the favoring of the microbial balance, caused by the presence of probiotic, paraprobiotics and postbiotics microorganisms or even the by-product of their metabolism (Barros et al. 2020; Dantas Coelho et al. 2020).

Great diversity of microbial species are used in the production of Kombucha; among which, acetic acid bacteria (AAB) belongs to the Acetobacteraceae family and yeasts are the majority. Currently, the AAB are classified into nineteen genera and several species (Gomes et al. 2018). The main bacteria reported in the literature are *Acetobacter* spp. (Sreeramulu et al. 2000), *Gluconacetobacter* spp. (Fu et al. 2014; Sreeramulu et al. 2000), and *Lactobacillus plantarum* (Fu et al. 2014). In addition, several species from genus Komagataeibacter, named in 2012, were isolated and sequencing from kombucha (Gomes et al. 2018, Laavanya, Shirkole & Balasubramanian, 2021). Regarding yeasts, *Candida Stellata*, *Rhodotorula mucilaginosa*, *Schizosaccharomyces pombe*, *Torulasporea delbrueckii* (Teoh et al. 2004), *Brettanomyces bruxellensis* (Chen and Liu 2000;

Teoh et al. 2004), *B. lambicus* (Mayser et al. 1995), *Saccharomyces cerevisiae* (Fu et al. 2014), and *Zygosaccharomyces Z. bailii* (Chen and Liu 2000; Teoh et al. 2004) are commonly reported.

Originally, the raw material most used in Kombucha preparation is the tea derived from the leaves of *Camellia sinensis*, a plant that belongs to the family of *Theaceae*. The use of the same plant with specific pre and post-harvest management, it is possible to obtain different varieties of tea, such as white, yellow, green, oolong, red and black (McGee 2004; Dantas Coelho et al. 2020). Traditional Kombucha uses preferably green or black tea. However, others varieties like oolong can also serve as a basis (Dufresne and Farnworth 2001; Watawana et al. 2016; Dantas Coelho et al. 2020). The main property attributed to the tea is the antioxidant capacity, an effect that can be explained by the polyphenols present in the form of catechins, which are considered secondary metabolites belonging to the family of flavonoids (Andersen and Markham 2006; Gomes et al. 2018). Regarding the herbs used, although *C. sinensis* is traditional, other species can be used as a base for beverage production. Thus, reports of Kombucha prepared with thyme (*Thymus vulgaris* L.), lemon verbena (*Lippia citriodora*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*), peppermint (*Mentha x piperita*), lemon balm (*Melissa officinalis* L.) or even coffee and coconut water have been presented in the literature (Battikh et al. 2012; Battikh et al. 2013; Četojević-Simin 2012; Watawana et al. 2015b; Watawana et al. 2016).

Ilex paraguariensis is a perennial tree native of South America and belongs to the family *Aquifoliaceae*. The distribution is prevalent in Argentina, Paraguay and Brazil, popularly known as yerba mate (Heck and De Mejia 2007). From *I. paraguariensis*, it is possible to produce different products by changing the processing stages of the leaves, such as crushing, drying and roasting.

The aim of this work was to develop and characterize non-alcoholic beverages fermented by mixed microbial culture with sour and refreshing sensorial profile, produced from the oolong tea, a traditionally herb used to produce Kombucha and the toasted yerba mate, a very popular tea in Brazil. This study presents the viability of producing Kombucha from yerba mate by comparing the physicochemical and sensorial parameters showed by the produced beverages.

2. Methodology

2.1 Material

Two different species of tea were used in the Kombucha preparing: oolong (*Camellia sinensis*) from China and toasted yerba mate (*Ilex paraguariensis* St. Hil.) from Brazil.

Starter culture (commercial Kombucha) was purchased from *Enlightened Kombucha Original*[®] - *GT's Synergy Drinks*[®] (Beverly Hills, EUA).

2.2 Preparation of Kombucha tea

For each tea infusion, 10% (w.v⁻¹) of organic sucrose solution (Native[®] - Sertãozinho, São Paulo - Brazil) was prepared with mineral water (Ouro Fino[®] - Campo Largo, Paraná – Brazil) and sterilized at 121°C for 15 min. Then, 8 g.L⁻¹ of infusion was added to this solution (85°C) and allowed to infuse for 10 min. The infusions were filtered and transferred to sterile glass jars (250 mL of tea per jar). The cool teas were inoculated with 10% (v.v⁻¹) of inoculum and fermented for 14 days at 30 °C.

Sampling was performed periodically at 0, 3, 7, 10 and 14 days of fermentation. 100 mL of the fermented tea was centrifuged at 10,000 rpm for 10 min and taken for the analysis. All the analyses were carried out in triplicate.

2.3 Physical chemical and chromatographic analysis

Total soluble solids (TSS) was measured in a digital refractometer (RM40, Mettler Toledo[®], USA) and expressed in °Brix (%). The pH was measured with an electronic pH meter (FE20 FiveEasy[®], Mettler Toledo[®], EUA). Total titratable acidity (TTA) was determined with 0.1 M NaOH solution (standardized) and phenolphthalein as indicator (AOAC, 2012). The acidity was expressed as acetic acid (g.L⁻¹), since this was the main organic acid present (see section 3.1). Alcohol content was determined by ebulliometer method (CTC, 2011).

Carbohydrates glucose, fructose and sucrose was determined by using Aminex HPX-87P column (9µm, 7.8 x 300 mm in ionic form Pb⁺², Biorad[®], EUA) at 80°C. The column was eluted with ultra-pure water (MilliQ[®], Millipore[®], EUA) as mobile phase at 1.0 mL.min⁻¹ flow rate. The photodiode array detector was programmed at a fixed wavelength of 215 nm and scan mode from 200 to 400 nm. Data acquisition and integration of chromatographic peaks were performed with the Shimadzu LCsolution[®] software. The samples were diluted in ultra-pure water (MilliQ[®], Millipore[®], USA) (1:2, v/v) and filtered (0.22 µm, Millipore[®], USA) before being injected into the system. Retention times were compared with analytical standards of sucrose, glucose and fructose (Sigma[®], USA).

Organic acids were determined in a HPLC system equipped with a photodiode array detector. For this, the samples were diluted (1:2, v/v) and filtered (0.45 µm, Millipore[®], USA) before being injected into the system. A MG C-18 column (5µ, 250 x 4.6 mm) (CapCell PaK, Shiseido[®], Japan) was used for the analysis. The mobile phase was a buffer 20 mM sodium phosphate (pH 2.4), maintained at 1.0 mL.min⁻¹ flow rate in isocratic elution. Detection was carried out at 215 nm. Data acquisition was performed using the Shimadzu LCsolution[®] software. The retention times of the chromatograms were compared with standards of acetic, succinic, oxalic, malic, citric, lactic and propionic acids (Sigma[®], USA).

2.4 Analysis of antioxidant and reducing capacity

Reducing capacity was determined by spectrophotometer method (Damiani et al. 2014). Absorbance was obtained at 760 nm and the results were expressed as mmol.L⁻¹ of Gallic Acid Equivalent (GAE) using the linear regression equation obtained from gallic acid calibration curve. Total flavonoids (TF) content was measured according Pękal and Pyrzynska (2014) modified. Briefly, 300 µL of 5% NaNO₂ followed by 0.5 mL of 10% AlCl₃ were added to 1 mL of sample and mixed. Samples were left for 5 min at room temperature in the dark, and then, 1 mL NaOH 1M was added. Absorbance was obtained at 510 nm and the results were expressed as mmol.L⁻¹ of Catechin Equivalent (CE) using the linear regression equation obtained from catechin calibration curve (0.05 – 0.2 mM). Antioxidant capacity on ABTS radical was determined on a UV-visible spectrophotometer (Thermo Electron Corporation[®], Spectronic Genesys 6[®], USA) at 730 nm (Re et al. 1999). The color control was prepared without the addition of the ABTS radical; the blank without the addition of sample and radical; and the control without addition of sample. Standard Trolox curve (1.0 to 8.0 mM) was constructed, and the results were expressed in mM equivalent of Trolox (mM Eq.Trolox). The percentage of inhibition rate of ABTS radical was calculated according to the follow:

$$\text{Inhibition \%} = \left\{ 1 - \left(\frac{\text{Abs sample}}{\text{Abs control}} \right) \right\} \times 100 \quad (\text{Eq. 1})$$

Determination of the antioxidant capacity on the radical DPPH was performed as follows: briefly, 50 µL of sample was mixed with 1 mL absolute ethanol, 1 mL acetate buffer (pH 5.5) and 0.5 mL DPPH 250 µM in ethanolic solution. It was homogenized and held for 15 minutes in the dark. Absorbance was determined at 517 nm on UV-visible spectrophotometer. Color control was prepared without addition of the DDPH radical; the blank without addition sample and radical; and the

control without addition of sample. Standard Trolox curve (0.2 to 1.0 mM) was constructed and the results expressed in mM equivalent of Trolox (mM Eq. Trolox). Percentage of inhibition rate of DPPH radical was also calculated according to Eq. 1.

2.5 Sensory evaluation of Kombucha

The samples at 7 and 10 days of fermentation were packaged in Amber bottles and maintained at 30 °C for 4 days. Then, gasified kombuchas were stored at 10 °C to perform the sensorial analysis. The method applied was the hybrid hedonic scale, consisting of a 10 cm line scale (0 = liked least; 10 = liked most), according to Villanueva et al. (2000). The test was carried out with a group of 100 untrained evaluators, aging over 18 years old, and both sexes. Samples were presented in a coded, randomized pattern, served in clear disposable cups (50 mL each at 10°C) and in individual booths lit with white light.

3. Results and Discussion

3.1 Physic-chemical characterization

Table 1 presents the results of TSS, pH and TTA. Both teas showed a significant decrease in TSS from 0 to 14 days of fermentation, since the reducing sugars were consumed by the microorganisms. Reducing carbohydrates were catabolized by microorganisms and converted to organic acids, causing a decrease in the pH of the beverages. The initial pH of Kombucha produced from oolong and yerba mate tea was 4.26 and 4.48, respectively. The samples exhibited a pH decrease that was progressive in relation to the fermentation time. At the finish time, they present a pH of 2.81 and 3.12, respectively. The observed pH values are similar with the results obtained by other authors, in whose works, different starter cultures and infusions of black and green tea resulted in beverages with a pH around 2.5 at the end of the fermentation time (Kallel et al. 2012; Sreeramulu et al. 2000). Between the 7th and 14th day of fermentation there was no significant difference in relation to the pH value for all the samples. This may be a consequence of the buffering effect between the synthesis of weak organic acids with minerals from the teas and water (Malbaša et al. 2011).

Table 1. Physicochemical results during fermentation time of Kombucha beverages produced from oolong and yerba mate

| Sample / Time (days) | TSS* (°Brix - %) | pH | TTA* g acetic acid.L ⁻¹ |
|-------------------------|---------------------------|--------------------------|---------------------------------------|
| Oolong | | | |
| 0 | 10.21 ± 0.04 ^a | 4.26 ± 0.37 ^a | 1.32 ± 0.29 ^d |
| 3 | 9.95 ± 0.05 ^b | 3.52 ± 0.16 ^b | 2.06 ± 0.34 ^d |
| 7 | 9.57 ± 0.06 ^c | 2.95 ± 0.09 ^c | 4.11 ± 0.48 ^c |
| 10 | 9.46 ± 0.00 ^d | 2.86 ± 0.03 ^c | 5.88 ± 0.48 ^b |
| 14 | 9.36 ± 0.03 ^e | 2.81 ± 0.09 ^c | 8.97 ± 0.88 ^a |
| Yerba Mate | | | |
| 0 | 10.24 ± 0.08 ^a | 4.48 ± 0.36 ^a | 1,03 ± 0.29 ^c |
| 3 | 10.14 ± 0.03 ^b | 4.00 ± 0.21 ^b | 1,32 ± 0.29 ^c |
| 7 | 9.82 ± 0.04 ^c | 3.42 ± 0.05 ^c | 2,94 ± 0.00 ^b |
| 10 | 9.67 ± 0.06 ^d | 3.31 ± 0.05 ^c | 3,82 ± 0.43 ^b |
| 14 | 9.52 ± 0.09 ^e | 3.12 ± 0.07 ^c | 6,76 ± 1.40 ^a |

Mean ± standard deviation (n = 3). Different lowercase letters on the same column indicate significant difference (Tukey, p <0.05).

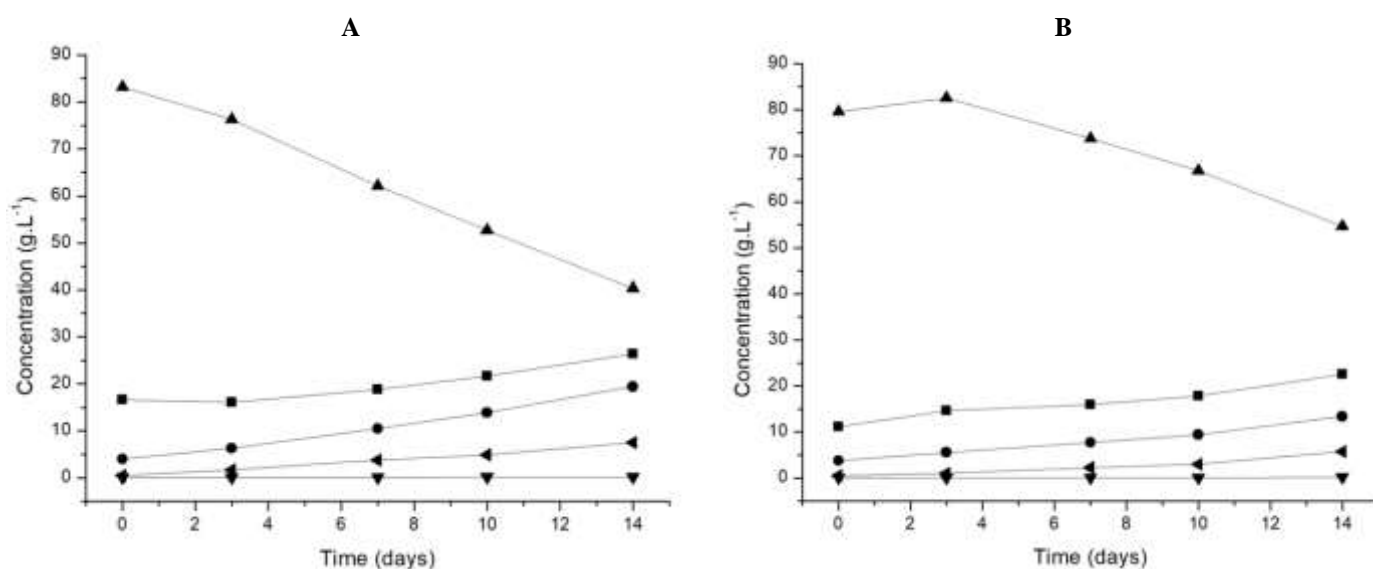
* Legend: TSS - Total Soluble Solids; TTA - Total Titratable Acidity.

Source: Authors.

There was an increase in TTA in all samples throughout the fermentation time. This can be explained by the increase in the concentration of organic acids, mainly acetic and succinic acid.

The sugars quantification (Figure 1) showed the occurrence of sucrose hydrolysis to glucose and fructose by the natural presence of enzyme invertase in both teas used (Chen and Liu 2000). Another possibility is that the invertase enzyme activity comes from the microorganisms present in the inoculum. The values obtained from the degradation rates of total sugars in formulation with oolong tea were $-1.72 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 3$ days); $-1.77 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 7$ days); $-1.56 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 10$ days); $-1.26 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 14$ days). Decreasing values were expected since the sugars are, in this case, the main source of carbon for maintenance and cell multiplication of the mixed culture.

Figure 1. Sugars and organic acids content of Kombucha beverages from (A) oolong and (B) yerba mate during fermentation time: (▲) sucrose; (■) glucose; (●) fructose; (◄) acetic acid; (▼) succinic acid.



Source: Authors.

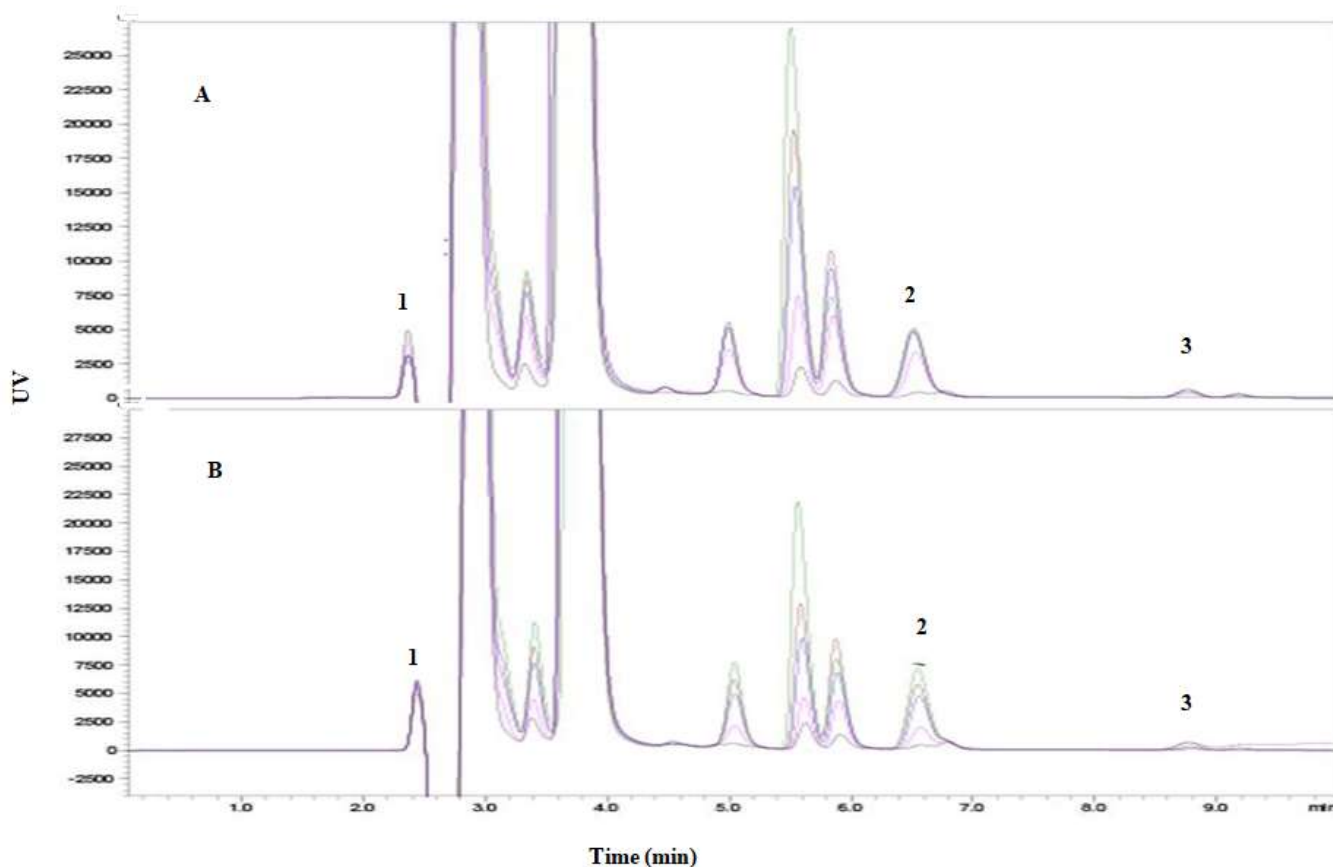
Total sugar content started from 103.85 g.L^{-1} and reached 86.15 g.L^{-1} at the end of the fermentation. According to the results, in Kombucha formulated with oolong tea, the accumulation of monosaccharides over the periods of 0 to 3; 0 to 7; 0 to 10 and 0 to 14 days, occurs with a formation rate of -0.16 ; 0.32 ; 0.51 and $0.70 \text{ g.L}^{-1}.\text{day}^{-1}$ glucose, respectively, and 0.76 ; 0.92 ; 0.99 and $1.10 \text{ g.L}^{-1}.\text{day}^{-1}$ fructose, respectively. The results showed that glucose was the primarily metabolized monosaccharide. The values obtained from the conversion rates of total sugars in Kombucha formulated with yerba mate tea were $2.75 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 3$ days); $-0.40 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 7$ days); $-0.07 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 10$ days); $-0.28 \text{ g.L}^{-1}.\text{day}^{-1}$ ($\Delta t = 14$ days). Total sugar content started from 94.65 g.L^{-1} ; at the finish time, it was 90.73 g.L^{-1} , which represents a decrease of 4%. In this same formulation, the formation rate of glucose ($\text{g.L}^{-1}.\text{day}^{-1}$) over periods of 0 to 3; 0 to 7; 0 to 10 and 0 to 14 days were 1.16 ; 0.67 ; 0.66 ; 0.81 , respectively. In relation to fructose, the values were 0.60 ; 0.56 ; 0.56 and 0.69 , respectively. Considering that, in this case, glucose was the monosaccharide accumulated in higher quantity, it was suggested that fructose was the main metabolized sugar by the microbial culture. It is known that microorganisms prioritize the route of glucose degradation. However, fructose can also be a source of energy for the cells when the enzyme system phosphotransferase and fructokinase is involved to fructose phosphorylation (Caescu et al. 2004).

Kallel et al. (2012) studied two types of Kombucha: green tea and black tea, prepared with an initial concentration of 100 g.L^{-1} sucrose and fermented for 15 days at $24 \text{ }^\circ\text{C}$. The beverage prepared with green tea had a sucrose degradation rate of $2.3 \text{ g.L}^{-1}.\text{day}^{-1}$ and, at the finish, were found contents of 5.2 g.L^{-1} glucose and 12.2 g.L^{-1} fructose. In the beverage prepared with black tea, the sucrose degradation rate at the finish time was $2.5 \text{ g.L}^{-1}.\text{day}^{-1}$ and the glucose and fructose contents were 9.4 g.L^{-1}

¹ and 17.8 g.L⁻¹, respectively.

The main organic acids responsible for conferring acidity to the Kombucha beverages were acetic and succinic acid (Fig. 2). Other acids such as oxalic, malic, citric, lactic and propionic have not been identified or could not be quantified. Acetic acid is a product of ethanol metabolism by acetic acid bacteria. The yeasts present in the microbial culture convert the sugar to organic acids, CO₂ and ethanol. Then, the produced ethanol is oxidized to acetic acid by the bacteria also present (Gomes et al. 2018). In the formulation prepared from oolong, acetic acid content ranged from 0.5 to 7.53 g.L⁻¹ and succinic acid from 0.02 to 0.15 g.L⁻¹ over 14 days of fermentation time. In the formulation prepared from yerba mate tea, the contents of acetic and succinic acid ranged from 0.54 to 5.75 g.L⁻¹ and 0.02 to 0.17 g.L⁻¹, respectively. The acetic acid concentration detected in both kombuchas also are stoichiometrically explained by the infusion preparation method, whose inoculum added already has acetic acid contents (as indicated in table 1 at time 0).

Figure 2. Organic acids chromatograms of oolong sample (a) and mate (b) during the fermentation. Day 0 (black); dia 3 (pink); day 7 (blue); day 10 (red); day 14 (green).



Source: Authors.

Bhattacharya et al. (2011) analyzed the profile of a Kombucha developed from the infusion of black tea and verified that the main acids present in the drink were acetic and gluconic acid. Jayabalan et al. (2014) report a diversity of organic acids such as acetic, gluconic, citric, L-lactic, malic, tartaric, oxalic and succinic acids present in Kombucha. The synthesis of these acids is attributed to the microbial action in the metabolization of sugars presents in the infusion. Glycolysis produces pyruvic acid and then, from decomposition of pyruvic acid can be formed acetic, lactic, propionic, succinic, malic and citric acid (in the cycle of tricarboxylic acids) (Sauer et al. 2008).

The alcohol content of both kombuchas ready to consume, were less than 0.1%. Therefore, the fermented teas were characterized as non-alcoholic beverages, according Brazilian legislation (BRASIL, 2009). This parameter is also used in other countries, although limits may vary from country to country.

3.2 Antioxidant capacity

Several *in vitro* assays were undertaken to highlight the potential antioxidant effects of the Kombucha beverages that might translate *in vivo*, since the teas used to prepare the infusions possess many secondary metabolites, such as flavonoids containing phenolic rings in their structure. The antioxidant capacity of flavonoids is due to their ability to sequester free radicals, thus reducing the potential for occurrence of chronic-degenerative diseases. In the *oolong* and yerba mate samples, reducing capacity did not differ significantly between the start and the finish of the 14 day-fermentation time (Table 2).

Table 2. Antioxidant capacity, flavonoids and total phenolic content during fermentation of Kombucha beverages from oolong and yerba mate.

| Sample / Time (days) | Reducing capacity mmol.L ⁻¹ GAE* | TF mmol.L ⁻¹ CE* | ABTS mEq Trolox | Inhibition rate of ABTS radical (%) | DPPH mEq Trolox | Inhibition rate of DPPH radical (%) |
|----------------------|---|-----------------------------|----------------------------|-------------------------------------|---------------------------|-------------------------------------|
| Oolong | | | | | | |
| 0 | 3.77 ± 0.49 ^{ab} | 0.595 ± 0.13 ^a | 6.252 ± 0.78 ^b | 71.72 ± 8.46 ^c | 0.949 ± 0.01 ^c | 85.75 ± 0.88 ^c |
| 3 | 4.12 ± 0.49 ^a | 0.499 ± 0.04 ^{ab} | 7.964 ± 0.36 ^a | 90.22 ± 3.91 ^a | 0.971 ± 0.01 ^b | 87.74 ± 0.57 ^b |
| 7 | 3.12 ± 0.15 ^b | 0.361 ± 0.02 ^{bc} | 6.401 ± 0.29 ^{bc} | 73.32 ± 3.12 ^{bc} | 0.973 ± 0.01 ^b | 87.96 ± 0.51 ^b |
| 10 | 4.11 ± 0.21 ^a | 0.414 ± 0.04 ^{bc} | 7.670 ± 0.45 ^a | 87.05 ± 4.91 ^a | 0.982 ± 0.00 ^a | 88.82 ± 0.37 ^{ab} |
| 14 | 3.57 ± 0.33 ^{ab} | 0.327 ± 0.02 ^c | 7.498 ± 0.48 ^{ac} | 85.19 ± 5.18 ^{ac} | 0.992 ± 0.01 ^a | 89.74 ± 0.45 ^a |
| Yerba Mate | | | | | | |
| 0 | 2.36 ± 0.97 ^a | 1.164 ± 0.40 ^a | 5.393 ± 1.37 ^a | 68.75 ± 9.60 ^a | 0.928 ± 0.02 ^b | 83.69 ± 1.71 ^b |
| 3 | 2.42 ± 0.12 ^a | 0.775 ± 0.08 ^b | 2.916 ± 0.45 ^b | 35.63 ± 4.85 ^b | 0.935 ± 0.01 ^b | 84.40 ± 1.15 ^b |
| 7 | 2.69 ± 0.28 ^a | 0.740 ± 0.13 ^b | 3.792 ± 0.79 ^{ab} | 45.11 ± 8.54 ^b | 0.930 ± 0.00 ^b | 83.91 ± 0.12 ^b |
| 10 | 2.87 ± 0.27 ^a | 0.665 ± 0.15 ^b | 3.385 ± 0.68 ^b | 40.71 ± 7.40 ^b | 0.941 ± 0.01 ^a | 84.99 ± 0.74 ^a |
| 14 | 3.31 ± 0.42 ^a | 0.612 ± 0.12 ^b | 3.347 ± 0.37 ^b | 40.30 ± 4.02 ^b | 0.960 ± 0.00 ^a | 86.72 ± 0.28 ^a |

Mean ± standard deviation (n = 3). Different lowercase letters on the same column indicate significant difference (Tukey, p <0.05).

* Legend: GAE - Gallic Acid Equivalent; TF - Total Flavonoids; CE - Catechin Equivalent.

Source: Authors.

Analyzing beverages prepared with green or black tea, Kallel et al. (2012) found an increase in the reducing capacity over 15 days of the fermentation. It was found an increase of 39% in green tea (from 4.584 to 6.388 mM Eq.AG) and 12% in black tea (from 5.936 to 6.583 mM Eq.AG). Similarly, Jayabalan et al. (2008) reported an increase of 19% in reducing capacity of green tea and 17% in black tea at the finish of the fermentation performed over 18 days. This increase may be related to the degradation of complex phenolic compounds (such as flavonoids) present in the tea samples, due to acid environment and the action of enzymes produced by the microorganisms present in the mixed culture (Jayabalan et al. 2007; Jayabalan et al. 2008).

Antioxidant capacity on the ABTS radical during the fermentation time (Table 2), demonstrated that the *oolong* sample at time 0 differed from the days 3, 10 and 14, indicating an increase in antioxidant capacity over the fermentation time. Regarding the inhibition rate on the radical ABTS (Table 2), the days 3, 10 and 14 did not differ from each other. However, they were significantly different from the time 0. In the yerba mate tea formulation, there was a decrease in the antioxidant capacity between the start and the finish of the fermentation time. At time 0 the highest values were found for both Trolox equivalence and ABTS radical inhibitory activity (5.393 mM and 68.75%, respectively). This situation can be attributed to the

initial culture, which provided phenolic compounds because it had an infusion of *C. sinensis* as basis. An antioxidant capacity of 3.357 mM Eq. Trolox and 40.30% of ABTS radical inhibitory activity were observed at the 14th day of fermentation.

Antioxidant capacity on the DPPH radical (Table 2) showed an increase during the fermentation time in both oolong and yerba mate samples. Trolox equivalent antioxidant activities increased 4.53% in oolong tea (from 0.949 to 0.992 mM Eq. Trolox) and 3.44% in yerba mate tea (from 0.928 to 0.960 mM Eq. Trolox). DPPH radical inhibitory activity (Table 2) for oolong and yerba mate tea at the start of the fermentation time were 85.75 and 83.69%, respectively, while at the finish time they increased to 89.74 and 86.72%, respectively.

The increase in antioxidant capacity can be explained by the fact that during Kombucha fermentation, the composition of bioactive compounds could be modified by the microbiota, since fermentation may have induced structural breakdown of plant cell walls. The decrease in pH observed over the fermentation creates condition for bound phenolic constituents to be released through enzymatic processes, which modifies the contents and structure of secondary metabolites and results in increase of the antioxidant capacity (Đorđević et al. 2010; Hunaefl et al. 2013)

Chu and Chen (2006) observed an increase in the inhibition rate of DPPH radical when analyzing Kombucha produced with 8 different mixed cultures and with the same infusion base of black tea. Among these mixed cultures, 4 presented rates of 61.2%; 64.3%; 69.2% and 62.2% starting at 34.3%. The other samples also increased during fermentation time, from 36.4%, to 47.8%; 39%; 43% and 49%. The authors considered that this increase was slower and suggest that the inhibitory activity of Kombucha may be associated with the mixed culture used.

3.3 Sensory evaluation

The profile of the evaluators was mostly female (66%) and young (18-26 years). As the evaluation was done in a university environment, it was expected that the occupation of the evaluators were students (91%) under graduating (65%).

Table 3 summarizes the scores of the evaluated attributes for 4 proposed beverage samples. In relation to the appearance, oolong fermented sample for 10 days differed significantly from the others, besides receiving the lowest score (6.2). The beverages had suspended particles from the biofilm produced by the mixed culture, which may have influenced the rating assigned by the judges.

Table 3. Sensory evaluation scores of kombuchas from oolong and yerba mate at different fermentation times.

| Sample | Attributes evaluated | | | | |
|---------------|----------------------|----------------------|-----------------------|---------------------------|-----------------------|
| | Appearance | Aroma | Flavor | Mouth-feel characteristic | Acceptability |
| Oolong Day 7 | 6.8±2.2 ^a | 4.5±2.6 ^a | 5.6±2.7 ^b | 5.9±2.8 ^a | 5.5±2.6 ^b |
| Oolong Day 10 | 6.2±2.3 ^b | 4.3±2.5 ^a | 5.2±2.9 ^c | 5.0±2.9 ^b | 4.7±2.7 ^c |
| Mate Day 7 | 6.9±2.1 ^a | 4.8±2.5 ^a | 6.4±2.3 ^a | 6.5±2.3 ^a | 6.3±2.2 ^a |
| Mate Day 10 | 6.9±2.1 ^a | 4.7±2.2 ^a | 6.2±2.6 ^{ab} | 6.5±2.5 ^a | 6.1±2.4 ^{ab} |

Mean ± standard deviation (n = 3). Different letters in the same column indicate significant difference (Tukey, p<0,05).

0: extremely disliked; 1: really disliked; 2: disliked moderately; 3: slightly disliked; 4: disliked; 5: did not like or dislike; 6: liked; 7: slightly liked; 8: liked moderately; 9: liked a lot; 10: extremely liked.

Source: Authors.

The attribute aroma did not present significant difference for any of the samples (p>0,05). The refusal of aroma may be justified by the presence of acetic acid, which has a detection threshold between 0.7-1.1 g.L⁻¹ in wines (European Commission 2012; Querol and Fleet 2006). Acetic acid content of the samples varied between 3.76-4.94 g.L⁻¹ in oolong and 2.31-3.04 g.L⁻¹ in yerba mate tea (time 7 and 10, respectively).

In relation to the flavor, oolong samples presented differences. The attributed notes were 5.6 and 5.2 for times 7 and

10 days, respectively, indicating a neutral response for the beverage. In contrast, the yerba mate samples did not present difference. However, the samples at time 7 were significantly different in relation to oolong samples, which reached a note of 6.4. This fact demonstrates that the evaluators enjoyed the acidic characteristic of the oolong fermented tea.

In relation to the mouth-feel attribute, oolong samples fermented for 10 days presented the lowest grade (5.0). The purpose of this evaluation was to appraise the feeling produced by the natural carbonation of the beverage. However, the flavor attribute may also influence due to the presence of organic acids produced by microbial action, since the longer fermentation time lead to the greater production of organic acids (Fig. 1). The results of the sensorial evaluation demonstrated that the evaluators preferred the less sour taste of the beverages, mainly in relation to oolong samples.

The acceptability for samples produced with yerba mate had average scores above 6, but no significant difference between the 7 and 10 days of fermentation. This suggests that the evaluators enjoyed the beverage. Oolong samples obtained the lowest averages, 5.5 and 4.7 for days 7 and 10, respectively. The preference of the evaluators for yerba mate Kombucha was probably due to the familiarity with the taste of the unfermented yerba mate tea by the evaluators, since in Brazil its consumption is widely diffused.

Since the evaluators were predominantly young (18-26 years) not accustomed to consuming fermented and sour products, the scores obtained for the evaluated attributes may have suffered negative interference. The sensory evaluation with trained evaluators using a Kombucha prepared from the infusion of oolong tea, Huang et al. (2015) demonstrated that on the 7 day of fermentation the highest acceptance of the beverage was obtained and, from the 8th to 11th day of fermentation, there was a significant decrease in acceptance.

4. Conclusion

Beverages formulated with oolong or yerba mate tea have been presented as an alternative in the production of Kombucha. It was found that the drink prepared with yerba mate was more accepted by the evaluators. The final beverage presented sensorial profile characteristic of Kombucha, acid and refreshing. However, the sensorial analysis showed that the aroma of acetic acid is not a desired aspect. So, 7 days of fermentation are sufficient for the development of the beverage.

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