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ANTIOXIDANT ACTIVITY OF COMMERCIAL AND TAILOR-MADE KOMBUCHAS AND THEIR NON-FERMENTED TEA

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ABSTRACT – Kombucha is a beverage obtained by the fermentation of *Camellia sinensis* tea and sugars by a symbiotic culture of microbiologically active bacteria and yeasts. Its consumption has become popular due to its possible functional properties, mainly its antioxidant capacity. However, because kombucha is not standardized and can have a wide range of compositions, it is challenging to demonstrate the health benefits of its consumption. We aimed at evaluating the antioxidant activity using the ORAC assay (Oxygen Radical Absorbance Capacity) of commercial and standardized green tea kombuchas and compared them with their unfermented tea. The ORAC results showed that standardized kombuchas did not differ significantly from non-fermented tea, while commercial kombuchas showed lower antioxidant capacity ($p < 0.05$). It is suggested then that the standardized starter culture used in tailor-made kombucha is a better technical choice to ferment kombucha while not impacting the amount of antioxidant compounds originally present in tea.

KEYWORDS: Functional beverages; Green tea; Bioactive compounds; Phytochemicals.

1. INTRODUCTION

Functional foods have gained interest from researchers, industries, and people due to their potential positive effects on health (Kapsak et al., 2011; Pimentel et al., 2021). Among the various functional beverages, kombucha has emerged as a popular fermented beverage in the food industry and is often produced at home. This fermented drink is produced by the fermentation of sweetened green and/or black tea decocts using a symbiotic microbiome known as Symbiotic Culture of Bacteria and Yeast (SCOBY) (Jayabalan et al., 2014).

The microbial composition of kombucha can differ significantly among producers, including commercial sources of the beverage (Chakravorty et al., 2016; Suhre et al., 2021; Fabricio et al., 2022). The microbial diversity of kombucha is important because it impacts the biochemical

composition of the beverage, resulting in changes in enzymes, vitamins, flavor, beneficial organic acids, alcohol content, and, as a consequence, the antioxidant activity. Thus, the use of a tailor-made starter culture could be a promising approach to ensure the standard composition, safety, quality, and functional properties of kombucha. Traditional kombucha is naturally carbonated, however, this method often results in alcohol levels that exceed the limits established by the Brazilian legislation. In this context, forced carbonation is a viable alternative for controlling and standardizing the production process.

Kombucha is recognized for its potent antioxidant properties, attributed primarily to tea-derived polyphenols (e.g., catechins, theaflavins, and thearubigins) and flavonoids (Vargas et al., 2021). During fermentation, these compounds undergo transformations that may either enhance or diminish their antioxidant capacity (Fabricio et al., 2022). To access these effects, this work aimed to evaluate the antioxidant activity of commercial and standardized green tea kombuchas – produced via forced and natural carbonatation - and compare them with their unfermented tea using the Oxygen Radical Absorbance Capacity assay (ORAC). This method of antioxidant analysis is robust, easy to reproduce and, uses conditions that are similar to those found in living organisms, such as a buffer pH of 7.4 and an equipment temperature of 37 °C.

2. MATERIAL AND METHODS

2.1 Samples of kombucha and tea

Five samples were used in this study: non-fermented tea, natural and forced carbonatation kombuchas (NC and FC, respectively), and two commercial brands of kombuchas (CB1- commercial brand 1, and CB2 – commercial brand 2). The tea sample used was organic green tea obtained from the company Vemat (SC, Brazil), and was prepared using 8 $g \cdot L^{-1}$ of the leaves added to boiling distilled water for 10 min. For preparation of tailor-made kombuchas, the same organic tea infusion was filtered through a membrane pore size of 0.22 μm and 50 $g \cdot L^{-1}$ of organic demerara sugar (Native, SP, Brazil) was added to the tea. After cooling, a tailor-made microbial starter culture composed of the bacterium *Komagataeibacter saccharivorans* (10^7 $CFU \cdot mL^{-1}$) and the yeasts *Brettanomyces anomala* (10^5 $CFU \cdot mL^{-1}$), and *Kluyveromyces marxianus* (10^6 $CFU \cdot mL^{-1}$) were added the infusion to start the fermentation process. The kombucha fermentation was performed in glass beakers at 28 °C for 48 h. The beakers were covered with sterile gauze and cheesecloth to provide an aerobic environment. For NC the fermented kombucha was bottled in a sterile hermetic glass bottle and a natural carbonation process was performed for more 48 h at 28 °C. For FC, we infuse CO_2 into the

kombucha from a 2 kg gas cylinder connected to a CO_2 regulator pressure gauge operating at 1 bar and a hose connection, similar to the process carried out in industries. The two commercial brands of kombucha were obtained from local commerce.

2.2 ORAC assay

The ability to inhibit peroxyl radicals was evaluated using the ORAC hydrophilic method described by Huang et al. (2005) (adapted). Tea and kombucha samples were diluted 1:200 in potassium phosphate buffer (75 mM), and Trolox was used as the control standard (from 8 to 96 $\mu mol \cdot L^{-1}$). Experiments were conducted in ELISA microplates with 150 µL of fluorescein solution (81 nM), 25 µL of the diluted sample or Trolox, and 25 µL of AAPH solution (152 mM). The assay was carried out on a fluorescence reader (Enspire 2300 Multimode Plate Reader, Perkin Elmer, USA), and readings were taken every minute for 90 min at 37 °C, with excitation and emission wavelengths of 485 and 528 nm, respectively. Results were calculated using the area under the curve (AUC) and expressed as µmol Trolox equivalents (TE) per mL of kombucha. The AUC was calculated by Equation (1) as:

$$AUC = 1 + \frac{f1}{f0} + \frac{f2}{f0} + \frac{f3}{f0} + \cdots \frac{fn}{f0} \quad (1)$$

where *f*0 was the initial fluorescence reading and *fn* was the fluorescence reading at each cycle.

2.3 Statistical analysis

The results obtained from ORAC assay were subjected to one-way analysis of variance (ANOVA), followed by Tukey post hoc test when necessary, using GraphPad PRISM® 10.0 software (Boston, MA, USA).

3. RESULTS AND DISCUSSION

Kombucha is considered a drink with high antioxidant potential, and this characteristic depends on the type and concentration of tea, fermentation time, and starter culture used (Jakubczyk et al., 2020; Malbaša et al., 2011). This potential is derived from tea, which is rich in catechins-theaflavin and tearubigin, characteristic that is the most related to the health benefits of kombucha (Cardoso et al., 2020; Jakubczyk et al., 2020). The results of antioxidant activity of the non-fermented broth, FC, NC, CB1, and CB2 are presented in Table 1.

Table 1. Oxygen radical absorbance capacity (ORAC) of non-fermented tea and NC, FC, CB1, and CB2 kombuchas.

	ORAC (μmol TE/mL)
Non-fermented tea	10.81 ± 1.47^{a}
NC	10.66 ± 0.80^{a}
FC	11.11 ± 0.55^{a}
CB1	8.22 ± 0.75^{ab}
CB2	5.13 ± 1.42^{b}

NC: natural carbonation; FC: forced carbonation; CB1: commercial brand 1; CB2: commercial brand 2. Different letters in the same column are significantly different as determined by the Tukey test ($p \leq 0.05$).

The fermentation can directly affect the antioxidant power of a product since this process involves the formation or bioconversion of bioactive compounds such as organic acids, responsible for combating reactive oxygen species (ROS) (Fabricio et al., 2022; Malbaša et al., 2011). The fermented kombuchas (FC and NC) did not differ significantly from the non-fermented tea, which is positive as green tea presents high antioxidant potential (Khan and Mukhtar, 2019). Also, it was possible to observe that natural carbonation, obtained in an anaerobic environment for an extra 48 h of fermentation, did not affect the ORAC values.

The results obtained for FC and NC showed that the synthetic starter culture using *K. marxianus fragilis*, *D. anomala*, and *K. saccharivorans* is suitable for kombucha fermentation and does not negatively impact the antioxidant compounds, as it may occur in some cases, as demonstrated by Malbasa et al. (2011), which used *S. cerevisae* and *Zygosaccharoyces* spp. as starter culture and observed a decrease in the antioxidant activity (measured through the DPPH radical). On the other hand, some studies have shown that fermentation may increase the antioxidant capacity of kombucha (Ahmed et al., 2020; Cardoso et al., 2020; Vargas et al., 2021). For example, Wang et al. (2020) developed a synthetic microbial community with *Acetobacter pasteurianus*, *Gluconacetobacter xylinus*, and *Zygosaccharomyces bailii* that resulted in an increase in the total phenol and flavonoid content during fermentation of kombucha, achieving maximum values in the day 8 of the process. Throughout the starter culture development in the present work, the kombucha fermentation period was reduced to 2 d (4 d in the case of NC kombucha), and the stability of the antioxidant power may be attributed to the short fermentation process.

The ORAC assay is a method that, in addition to being adaptable for several matrices, also is relevant to *in vivo* conditions, as it measures the radical chain-breaking ability of antioxidants using a peroxyl radical, which is the predominant free radical in human biology (Prior, 2015; Zhong and Shahidi, 2015). However, only a few studies have assessed the kombucha antioxidant capability using this assay. Fabricio et al. (2022) found ORAC values from 8.1 to 9.4 μmol/mL in kombuchas fermented from 8 g/L of green tea for 5 d, whereas Sun et al. (2015), fermented kombucha using 10 g/L of black tea for 12 d and found values of approximately 4 to 6.42 μmol/mL. Silva Junior et al. (2021) also evaluated the potential antioxidant of kombucha by ORAC assay. They found even lower values, varying from 1.82 to 3.71 μmol/mL, in traditional kombucha fermented for 7 d using 125 g/L of green tea as substrate. All those works that performed the ORAC analysis in kombuchas presented lower amounts in comparison to FC and NC kombuchas obtained in this work. Besides CB1 and CB2 labels showing they were also produced from green tea, the CB2 presented the lowest antioxidant capacity and differed ($p < 0.05$) from FC and NC. This difference may be related to the microorganisms involved in the fermentation process of CB1 kombucha and possibly to the concentration of green tea used in the infusion by producers.

Since kombucha and green tea exhibit comparable antioxidant activity, kombucha may offer additional health benefits due to its fermentation-derived metabolites - such as organic acids - as well as live microorganisms. Moreover, kombucha's effervescent quality and tangy flavor profile make it a more appealing functional beverage for many consumers compared to traditional green tea.

4. CONCLUSION

The ORAC results show that standardized kombuchas did not differ significantly from non-fermented tea, while commercial kombuchas showed the lowest antioxidant capacity ($p < 0.05$). Therefore, the synthetic starter culture used in tailor-made kombucha is suitable for kombucha's fermentation and does not impact the antioxidant compounds of tea.

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