

# Influence of the addition of strawberry guava (*Psidium cattleianum*) pulp on the content of bioactive compounds in kombuchas with yerba mate (*Ilex paraguariensis*)

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## Abstract

Kombucha is a beverage obtained by fermenting tea with a symbiotic culture of bacteria and yeasts. It has been investigated to explore less-used raw materials added to kombucha to partially replace tea and flavor the drink. This influences the sensory characteristics and the concentration of bioactive compounds. In this context, this study aimed to evaluate the influence of adding strawberry guava pulp on the total phenolic compound (TPC) content and *in vitro* antioxidant capacity of kombuchas made with green tea and yerba mate. Thus, it was observed that the fermentative process resulted in increased TPC content in the formulations made with green tea (T1) and with green tea and yerba mate (1:1) (T3). Furthermore, these formulations flavored with strawberry guava pulp presented the highest TPC contents [184.39 and 150.78 mg gallic acid equivalent (GAE)/100 mL, respectively]. Furthermore, the formulation T1 showed a high antioxidant capacity for ABTS<sup>+</sup> and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. On the contrary, the formulation T3 added with strawberry guava pulp showed the highest antioxidant capacity (5.24  $\mu$ M TEAC/mL) by the ferric reductive antioxidant potential (FRAP) method. Thus, the strawberry guava pulp proved to be a promising alternative for the flavoring and diversification of kombuchas.

**Keywords:** tea fermentation; functional beverage; antioxidant capacity; phenolic compounds.

**Practical Application:** Yerba mate and strawberry guava have potential applications in the diversification and bioactive compound contents of kombuchas.

## 1 INTRODUCTION

The demand for new beverages, exceptionally functional beverages, is a trend in the food industry (Rocha-Guzmán et al., 2023). Among them, kombucha has been highlighted. This fermented beverage is made from tea (*Camellia sinensis*), sugar, and a symbiotic culture of bacteria and yeast (SCOBY). Consumers' growing interest in this beverage is related to its various functional properties, such as anti-inflammatory potential and antioxidant activity (Freitas et al., 2022). Consequently, it is estimated that the global Kombucha market will reach \$ 11.8 billion by 2030, with a compound annual growth rate (CAGR) of 16.8% between 2022 and 2030 (Global Industry Analysts, 2023).

Traditionally, this beverage is made using green or black tea (Freitas et al., 2022). However, research reports the use of other types of raw materials as partial substitutes for commonly used ingredients, such as guava (Khaleil et al., 2020), soursop (Tan et al., 2020), goji berry (Abuduaibifu & Tamer, 2019), and mustard leaves (Rahmani et al., 2019) among others. Despite the broad diversification of raw materials for kombucha production, the effect of alternative raw materials on the flavoring step still needs to be explored.

In this perspective, native Brazilian species show potential for partially replacing traditional kombucha ingredients and for the beverage flavoring step. A promising alternative is partially substituting green tea with a regional raw material, such as yerba mate (*Ilex paraguariensis*). Yerba mate is usually added to different food products as a natural antioxidant (Santetti et al., 2021) and is culturally important in Brazil. Moreover, considering that native fruits are a good source of biologically active compounds, strawberry guava (*Psidium cattleianum*) stands out as a raw material with high potential to diversify and flavor kombucha due to its richness in phenolic compounds, vitamin C, and, consequently, high antioxidant capacity (Pereira et al., 2018).

In this sense, the partial replacement of green tea with yerba mate in association with the strawberry guava flavoring step becomes an alternative for diversifying kombuchas, promoting the addition of value and the possibility of strengthening the production chain of these raw materials. Thus, this study aimed to evaluate the influence of adding strawberry guava pulp in relation to fermentative parameters, total phenolic compound (TPC) content, and *in vitro* antioxidant capacity in kombuchas made with green tea and yerba mate at different production stages.

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## 2 MATERIALS AND METHODS

### 2.1 Raw materials and reagents

The fruits of strawberry guava were collected in the 2021 harvest in the municipality of Lages in Santa Catarina. The fruits were sanitized with a sodium hypochlorite solution (100 mg/L) and were then pulped in an electric pulper (model DP-50, Tomasi, São Paulo, Brazil).

The gallic acid, ABTS<sup>+</sup> [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, TPTZ [2,4,6-Tris(2-pyridyl)-s-triazine], Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2 Kombucha elaboration

The preparation consisted of the traditional kombucha production methodology (Leonarski et al., 2022). Initially, an inoculum was prepared, which consisted of the infusion of 0.05% of green tea (*Camellia sinensis* (L.) Kuntze) at 80°C for 10 min, and sucrose (5% w/v) and the 14-cm diameter SCOBY (Companhia dos Fermentados, São Paulo, Brazil) were then added, followed by a fermentation (14 days) to pH 3.5. Next, a control formulation (T1) was prepared with 1% green tea, and two formulations with partial replacement of green tea (Amay Chás, São Paulo, Brazil) with yerba mate (*Ilex paraguariensis*) (Serrana Nativa, Santa Catarina, Brazil): with 0.75% green tea and 0.25% yerba mate (T2) and with 0.5% green tea and 0.5% yerba mate (T3). All formulations were prepared by infusion at 80°C for 10 min, and then sucrose (5% w/v) and inoculum (10% v/v) were added. Finally, the fermentative process was performed in glass fermenters in a BOD incubator (SOLAB SL-200/90) at 20°C for 9 days. At the end of the fermentation, the kombuchas were filtered and then flavored by blending based on preliminary tests. This way, 10% (v/v) of strawberry guava pulp was added in all three treatments, and the samples were kept refrigerated at 4°C for 9 days.

### 2.3 Physicochemical parameters

Physicochemical analyses of the kombucha were performed at different stages (pre-fermentation, fermentation, post-fermentation, and post-flavoring). The pH was determined using a potentiometer (AKSO AK90), while the total soluble solids content (°Brix) was determined with an analog refractometer (AKSO RHB32). Total acidity (g acetic acid/L) was evaluated according to the methodology established by AOAC (2005). The alcohol content of the flavored kombucha sample was determined using an electronic enochemical distiller (Gibertini®).

### 2.4 Total phenolic contents and *in vitro* antioxidant assay

TPC content and *in vitro* antioxidant capacity were determined at different process stages (pre-fermentation, post-fermentation, and post-flavoring). The TPC content was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965). The absorbance reading was performed at 765 nm in a UV-VIS

spectrophotometer (model U-1800, Hitachi, Japan), and the results were expressed as mg gallic acid equivalent (GAE) per 100 mL of sample. The antioxidant capacity was determined by the free radical scavenging activity (ABTS<sup>+</sup>) (Re et al., 1999), DPPH stable radical sequestrant capacity (Brand-Williams et al., 1995), and the ferric reductive antioxidant potential (FRAP) method (Benzie & Strain, 1996). The results of the assays were expressed as μM Trolox equivalent (TEAC)/mL of the sample.

### 2.5 Statistical analysis

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's test ( $p \leq 0.05$ ) were performed using the OriginPro® version 2022 software.

## 3 RESULTS AND DISCUSSION

### 3.1 Physicochemical parameters

During the fermentation process of the kombuchas, the pH, total soluble solids content, and acidity were evaluated (Figure 1). The fermentation contributed to the decrease ( $p \leq 0.05$ ) of the pH values in all samples evaluated. Furthermore, the lowest pH values were observed in the samples with partial replacement of green tea by yerba mate (T2 and T3). This performance is associated with the production of organic acids as it is intensified by the culture present in the SCOBY during the fermentation process (Yuliana et al., 2023). As a result, the acidity values increased with decreasing pH, ranging between  $1.58 \pm 0.00$  and  $2.88 \pm 0.00$  g acetic acid/L.

The TSS content of all samples had the same behavior during the fermentation process, and there was a reduction of around 10% in the TSS content (Figure 1). The changes in TSS content may be caused by the conversion of sucrose into glucose and fructose through hydrolysis. These sugars are then metabolized to promote microbial growth and the production of metabolites (Sharifudin et al., 2021). Thus, the partial substitution of yerba mate for green tea did not affect the conversion of sugars.

In the different stages (pre-fermentation, post-fermentation, and post-flavoring), it was observed that there was variation ( $p \leq 0.05$ ) in the parameters evaluated (Table 1). As seen previously, the fermentative process influenced the characteristics of the kombuchas. However, adding strawberry guava pulp increased ( $p \leq 0.05$ ) pH, TSS, and acidity values. This behavior is related to the characteristics of strawberry guava pulp, which had a pH of 3.0 and TSS of 8.8°Brix. According to Pereira et al. (2018), the physicochemical parameters of strawberry guava pulp influence sensory characteristics, resulting in a sweet-to-sour pulp and a significant ascorbic acid content.

In this sense, the addition of 10% (v/v) strawberry guava pulp resulted in kombuchas with a TSS content ranging from  $5.00 \pm 0.00$  to  $5.20 \pm 0.00$  °Brix and an acidity ranging from  $2.48 \pm 0.10$  to  $3.28 \pm 0.10$  g acetic acid/L. Furthermore, in a study conducted by Zubaidah et al. (2018), kombuchas with higher TSS contents showed higher flavor scores than samples

containing lower TSS contents. Thus, kombuchas flavored with strawberry guava may be an attractive alternative for consumers seeking a balance between sweetness and acidity.

### 3.2 Total phenolic contents and *in vitro* antioxidant assay

The TPC contents (Figure 2) ranged from 64.67 to 184.39 mg GAE/100 mL. The results found in this study were higher than those reported by Zubaidah et al. (2018), who, when evaluating the TPC content of kombuchas made with Salak Suwaru fruits, observed a range between 27.50 mg GAE/100 mL (pre-fermentation) and 62.30 mg GAE/100 mL (post-fermentation). It is also observed that the treatment T2, after fermentation (T2.1), was the one that showed the lowest content of TPC, not having a positive effect, the addition of 0.25% yerba mate in the formulation of kombucha.

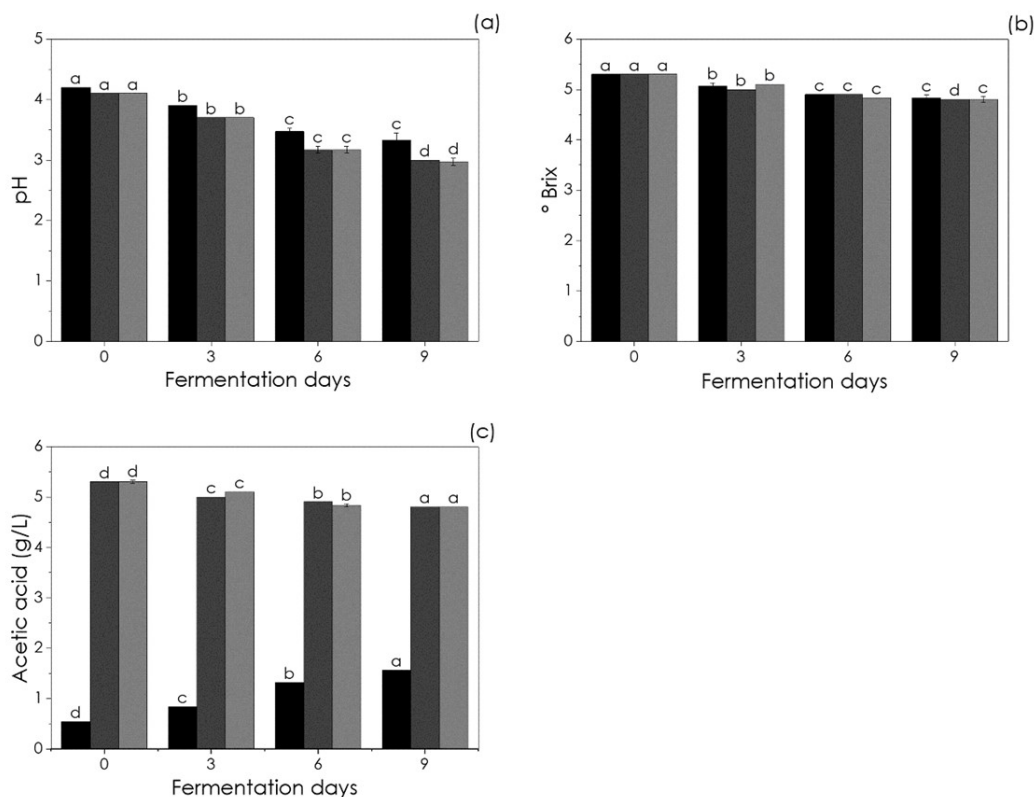
The fermentation process of T1 and T3 kombuchas resulted in increased TPC ( $p \leq 0.05$ ). Other studies have reported this behavior as fermentation releases phenolic compounds (Gamboa-Gómez et al., 2016; Zubaidah et al., 2018). Moreover, it was observed that the addition of strawberry guava pulp positively influenced ( $p \leq 0.05$ ) the TPC content as the formulations T1 and T3 flavored with strawberry guava pulp showed the highest TPC contents,  $184.39 \pm 7.09$  and  $150.78 \pm 1.27$  mg GAE/100 mL, respectively. Studies have reported the bioactive potential of strawberry guava by its chemical composition, which is mainly composed of phenolic compounds, flavonoids, and anthocyanins (Medina et al., 2011; Pereira et al., 2020).

As for the *in vitro* antioxidant capacity results (Figure 3), it was observed that the free radical scavenging activity (ABTS<sup>+</sup>) method showed the most expressive values ( $p \leq 0.05$ ), ranging between 13.17 and 18.99  $\mu\text{M TEAC/mL}$ . Furthermore, sample T1 showed a high antioxidant capacity *in vitro*, which remained stable ( $p > 0.05$ ) in the different process steps, according to the ABTS<sup>+</sup> and DPPH methods. This high antioxidant capacity of kombuchas is attributed to both the raw material used and the metabolites produced by the cultures present in SCOBY (Yuliana et al., 2023). Sample T1 shows a high antioxidant capacity due to using green tea as the initial raw material. Furthermore, according to Jakubczyk et al. (2020), when comparing kombuchas made from different types of tea, it was found that green tea stood out for its higher antioxidant potential.

**Table 1.** pH, TSS (°Brix), and acidity (g acetic acid/L) of the samples in the pre-fermentation, post-fermentation, and post-flavoring stages.

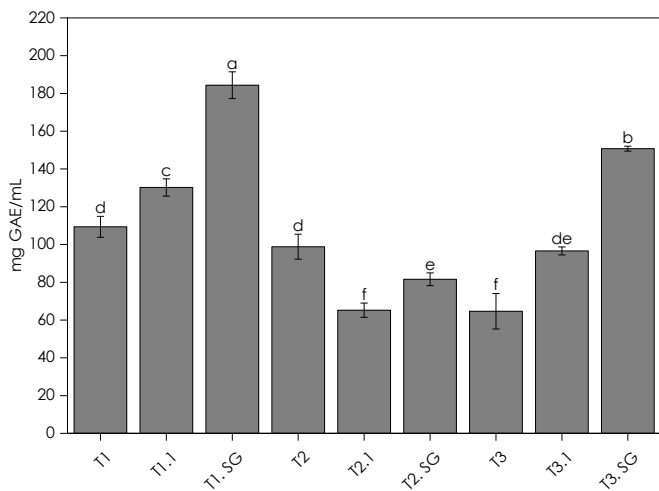
Samples	pH	Total soluble solids	Acidity
Pre-fermentation	T1	$4.20 \pm 0.00^a$	$5.30 \pm 0.00^a$
	T2	$4.10 \pm 0.00^a$	$5.30 \pm 0.00^a$
	T3	$4.10 \pm 0.00^a$	$5.30 \pm 0.00^a$
Post-fermentation	T1	$3.33 \pm 0.12^b$	$4.83 \pm 0.06^d$
	T2	$3.00 \pm 0.00^c$	$4.80 \pm 0.00^d$
	T3	$2.97 \pm 0.06^c$	$4.80 \pm 0.00^d$
Post-flavoring	T1	$3.40 \pm 0.10^b$	$5.20 \pm 0.00^b$
	T2	$3.20 \pm 0.10^b$	$5.00 \pm 0.00^c$
	T3	$3.30 \pm 0.10^b$	$5.00 \pm 0.00^c$

<sup>a-c</sup>Different letters in the same column indicate statistical differences ( $p \leq 0.05$ ) between the samples. These were T1 (1% green tea), T2 (0.75% green tea and 0.25% yerba mate), and T3 (0.5% green tea and 0.5% yerba mate).



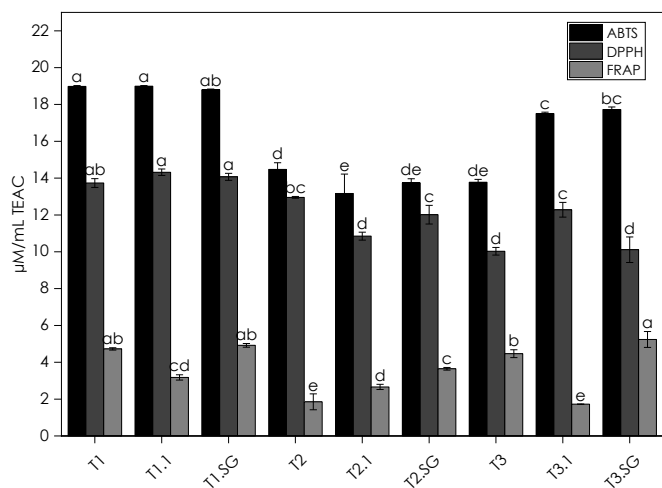
<sup>a-d</sup>Different letters in the different columns indicate significant differences ( $p \leq 0.05$ ) between fermentation days for the same sample ( $n = 3$ ); ■: T1; ■: T2; ■: T3.

**Figure 1.** (a) pH, (b) TSS (°Brix), and (c) acidity (g acetic acid/L) during the fermentation process of kombucha samples.



<sup>a-d</sup>Different letters indicate significant differences between samples ( $p \leq 0.05$ ). These were T1 (1% green tea), T2 (0.75% green tea and 0.25% yerba mate), and T3 (0.5% green tea and 0.5% yerba mate).

**Figure 2.** Total phenolic compound content of kombucha samples at pre-fermentation (T1, T2, and T3), post-fermentation (T1.1, T2.1, and T3.1), and post-flavoring (T1.SG, T2.SG, and T3.SG).



<sup>a-d</sup>Different letters indicate significant differences between samples ( $p \leq 0.05$ ) for the same method. These were T1 (1% green tea), T2 (0.75% green tea and 0.25% yerba mate), and T3 (0.5% green tea and 0.5% yerba mate).

**Figure 3.** *In vitro* antioxidant capacity of kombucha samples at pre-fermentation (T1, T2, and T3), post-fermentation (T1.1, T2.1, and T3.1), and post-fermentation (T1.SG, T2.SG, and T3.SG).

The *in vitro* antioxidant capacity results of this study by the ABTS<sup>+</sup> and DPPH methods were higher than those reported by Yildiz et al. (2020) when evaluating kombuchas made with varieties of black carrots found an average antioxidant capacity of 2.94 and 3.70  $\mu\text{M TEAC/mL}$  for the ABTS<sup>+</sup> and DPPH methods, respectively.

The formulation T3, containing strawberry guava pulp, showed the FRAP method's highest antioxidant capacity *in vitro* ( $5.24 \pm 0.43 \mu\text{M TEAC/mL}$ ). However, no statistically significant differences were found when compared with formulation T1 in both the pre-fermentation and post-flavoring stages ( $p > 0.05$ ). This behavior may be related to the compounds responsible for the antioxidant activity present in green tea and yerba mate.

Gerolis et al. (2017) evaluated extracts of green tea and yerba mate and found higher antioxidant activity in green tea by the ABTS<sup>+</sup> and DPPH methods, while yerba mate stood out in the FRAP method.

The results obtained in this study revealed a significant effect ( $p \leq 0.05$ ) of adding strawberry guava pulp in combination with the partial substitution of green tea for yerba mate on the *in vitro* antioxidant capacity, evaluated by the FRAP method. Specifically, there was an increase of approximately 97% in antioxidant capacity for the T2 formulation samples and an increase of approximately 17% for the T3 formulation samples. They indicated a positive impact on the *in vitro* antioxidant capacity of the final product. The addition of strawberry guava pulp may have contributed to this increase, possibly due to bioactive compounds, such as polyphenols and vitamin C, found naturally in the fruit (Pereira et al., 2018).

#### 4 CONCLUSION

The results of this study showed that the strawberry guava pulp contributed to the increase of TPCs in kombuchas made with green tea, and green tea and yerba mate (1:1). Furthermore, it was concluded that the partial replacement (50%) of the green tea with yerba mate showed satisfactory results for the content of TPCs and antioxidant capacity *in vitro*.

From this study, it can be concluded that adding strawberry guava pulp as a way of flavoring kombucha presents a potential in the diversification of this beverage, aiming for the valorization and conservation of this species, besides positively influencing the chemical characteristics of the product.

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