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Optimization of yeast reuse in beer production through the use of *Camellia sinensis* leaves

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Abstract

Beer and green tea (*Camellia sinensis*) are among the most consumed beverages worldwide. In brewing processes, the reuse of yeast slurry is a common practice, which can occur over several generations as long as cell vitality is maintained. Given that green tea contains a high concentration of antioxidant compounds, this study evaluated the effect of partially replacing hops with *C. sinensis* leaves during the whirlpool stage to optimize yeast vitality over successive reuses. Summer ale beers were produced on a laboratory scale (0.7 L), with hop substitutions of 20 and 40% using *C. sinensis* leaves. Yeast vitality was assessed over five yeast generations through the acidification power test, and antioxidant activity was measured using the 2,2-Diphenyl-1-picrylhydrazyl assay. The results showed that 20% maintained yeast vitality and improved wort attenuation, pH, and beer color parameters without compromising sensory characteristics. Conversely, the 40% substitution led to undesirable changes, particularly in color, flavor, and acid balance, offering no technological advantages. It is concluded that a 20% partial replacement of hops with green tea leaves is a promising alternative for producing beer with enhanced fermentation stability.

Keywords: green tea; hops; wort; acidification power; antioxidant activity.

Practical Application: The craft and industrial brewing industry can adopt the partial replacement of 20% of hops with green tea leaves (*Camellia sinensis*) during the whirlpool phase.

1 INTRODUCTION

Beer is one of the oldest beverages in the world and ranks among the most consumed alcoholic beverages globally, representing a market with vast investment and research opportunities (Gobbi et al., 2024). In 2024, the sector generated approximately US$851.15 billion in the global economy (Fortune Business Insights, 2024), highlighting its significant commercial importance and consumer interest. In recent years, consumers have become more discerning in their choice of beer, seeking not only high quality but also sensory differentiation and innovative experiences, and they are willing to pay more for specialty beers (Bimbo et al., 2023).

The loss or reduction of yeast vitality in the brewing process is exacerbated by high concentrations of free radicals, which damage cellular structures at various levels and organelles. As a result, cultures with low vitality become unsuitable for multiple cycles of yeast slurry reuse, a common practice in the brewing industry. Under these conditions, yeast struggles to initiate wort fermentation, leading not only to delays in the fermentation process but also to the formation of undesirable sensory characteristics in the beer due to increased production of byproducts such as aldehydes and ketogenic compounds. Therefore, ensuring appropriate conditions to maintain yeast vitality and viability is essential to preserve the quality of the final product and enable cost reduction through inoculum reuse (Telini et al., 2020).

Yeast reuse, typically collected from the bottom of conical tanks, is a widespread practice in the brewing industry, as it significantly reduces production costs by eliminating the need for fresh cultures in each wort batch. After collection, yeast can be immediately reused or stored frozen for future use. However, the number of reuses is limited, as successive cycles negatively impact yeast vitality and viability. Using successive generations from a pure culture (freshly acquired yeast) can cause cellular damage, impair replication, and lead to macromolecule oxidation, consequently affecting the quality of the produced beer (Garge et al., 2024).

In contrast, tea is another widely consumed beverage, particularly in Eastern countries, with its most popular form derived from the infusion of *Camellia sinensis* leaves. Green tea is rich in phenolic compounds, which exhibit high antioxidant activity, particularly catechins, accounting for approximately 6–16% of the dry leaf mass (Camargo et al., 2016; Gong et al., 2020).

The antioxidants in *C. sinensis* are capable of mitigating oxidative stress through various mechanisms, such as inhibiting

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pro-oxidant enzymes, scavenging free radicals, and chelating metal ions. Due to its high catechin content, green tea is a significant natural source of antioxidants, acting as a reducing agent or metal chelator (Camargo et al., 2016; Koren et al., 2019).

According to Guglielmotti et al. (2020), the main factors limiting beer shelf life are oxidation and turbidity. Oxidation leads to the formation of undesirable volatile compounds (off-flavors). However, the presence of polyphenols can mitigate these effects by neutralizing free radicals, a process mediated by the phenolic hydroxyl group, which stabilizes the unpaired electron in its aromatic ring.

Given the presence of antioxidant compounds in *C. sinensis* leaves, this study aims to evaluate the effectiveness of adding ground green tea during the boiling stage of beer production, investigating whether its antioxidant activity can protect yeast from free radicals produced during its metabolism. This approach aims to enhance cellular vitality, allowing for the reuse of yeast cultures for up to five additional fermentation cycles.

1.1 Relevance of the work

The relevance of this study lies in the search for innovative and economically viable solutions to maintain yeast vitality over multiple reuse cycles in beer production. The partial replacement of hops with *Camellia sinensis* leaves emerges as a promising alternative, as this ingredient is widely available, lower in cost, and provides antioxidant compounds capable of mitigating oxidative damage typical of fermentative metabolism. Thus, the study contributes to improving fermentation efficiency, reducing operational costs, and increasing process stability, offering a novel and applicable approach for both craft and industrial breweries.

2 MATERIAL AND METHODS

2.1 Camellia sinensis sample

Green tea leaves were purchased locally (Maringá, PR, Brazil) and ground before adding them to the beer. The leaves were stored in airtight containers, protected from sunlight and moisture.

2.2 Beer production process

The beers were produced in Maringá, PR, at the State University of Maringá, Brazil. The summer ales were brewed with a standard formulation for all beer samples, as shown in the Table 1:

Table 1. Summer Ale beer formulation.

Ingredients	Quantity
Malt—Pilsen	2.5 kg
Malt—Munich II	0.8 kg
Wheat Malt	0.5 kg
Malt—Carapils	0.3 kg
Hops—Hallertau Magnum (Boil 60 min)	15 g
Hops—Cascade (Whirlpool 30 min)	40 g
Hops—Ella (Whirlpool 30 min)	25 g

2.3 Leaf substitution

The leaves were added during the wort boiling stage to ensure proper extraction of antioxidant compounds. The substitutions followed the concentrations listed below (Table 2), replacing the bittering hops Hallertau Magnum:

Six generations of beer were produced, with each generation including three variations (control, low, and high concentration), as shown in Table 2. Each variation was made in triplicate in 0.7 L volumes, using the residual yeast slurry from the previous batch for the subsequent beer production.

2.4 Yeast vitality

Yeast culture vitality was evaluated based on the acidification power (AP) test, following the method by Kaka et al. (1988) with adaptations. A 3 mL aliquot of each yeast slurry sample was centrifuged at 9000 rpm for 3 min at 25 °C. The cell precipitate was washed three times with sterile distilled water and then mixed with 5 mL of sterile distilled water (pH 6) at room temperature under magnetic stirring. The initial pH of the cell suspension was recorded, followed by another reading after 10 min. Then, 500 µL of a sterile 50% (w/v) glucose solution was added, and the pH was re-recorded after 10 min of stirring. The AP, which indicates yeast vitality, was calculated using Equation 1:

$$PA = (pH0 - pH10) + (pH10 - pH20) \quad (1)$$

Where pH0 is the initial pH (time = 0 min), pH 10 is the pH after 10 min, when glucose was added, and pH20 is the pH after 20 min (10 min after the addition of glucose).

2.5 Yeast viability

Viable cells were assessed using the methylene blue citrate staining method, as described by Smart et al. (1999). Live yeast cells remain unstained, while non-viable cells are stained blue. An aqueous solution of methylene blue (0.1% w/v) was used for staining. Yeast cells were diluted in 0.1 M glycine buffer at a 1:100 ratio, and 1 mL of the dilution was incubated with 1 mL of methylene blue for 15 min. Stained (non-viable) and unstained (viable) cells were counted in a 10 µL aliquot of this suspension using a Neubauer chamber.

Sample viability was determined using Equation 2:

$$Viability\ (\%) = \frac{\sum tc - \sum cm}{\sum tc} \times 100 \quad (2)$$

Table 2. Concentrations of *Camellia sinensis* leaf substitutions.

Beer sample	Leaf concentration (%)
Control	0
Low concentration	20
High concentration	40

Where ∑ tc is the total count in the Neubauer chamber and ∑ cm is the count of non-viable (stained) cells in the Neubauer chamber.

2.6 Determination of total phenolic compounds in beer

Total phenolic compounds were determined using the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965) with modifications (Zhao et al., 2010). The technique involved mixing 0.5 mL of diluted beer with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent, allowing a 5-min reaction, followed by the addition of 2 mL of 7.5% Na_2CO_3 and adjusting the volume to 10 mL with deionized water. After 30 min at room temperature, absorbance was measured at 760 nm. The absorbance was compared to a gallic acid standard curve, and the results were expressed as milligrams of gallic acid equivalent per liter of beer (mg GAE/L).

2.7 Antioxidant activity

The free radical scavenging activity of the beers was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Rufino et al. (2010).

2.8 Beer color

Color analysis was performed according to the American Society of Brewing Chemists Methods of Analysis (2015). Values were reported in European Brewery Convention (EBC) units using a spectrophotometer at a wavelength of 430 nm.

2.9 Statistical analysis

Data were evaluated using analysis of variance, and means were subjected to Tukey's test at a 5% significance level using the Statistica software.

3 RESULTS AND DISCUSSION

3.1 Attenuation and vitality

The percentage of attenuation and yeast vitality was assessed. The results are presented in Table 3.

The specific gravity of the fermented beer was measured to evaluate the degree of metabolization of fermentable sugars. It is known that yeast with good vitality, in a minimally favorable environment, metabolizes a greater amount of these carbohydrates, reducing the wort's specific gravity and producing ethanol, carbon dioxide, and other byproducts. Table 3 shows statistical consistency in attenuation percentages for productions without leaf addition across yeast reuse cycles. In contrast, with 20% leaf addition, there is an increase in attenuation from R0 onward, suggesting improved yeast vitality and long-term adaptation across generations. For the 40% leaf addition treatment, an increase in attenuation rate is observed from the second reuse (R2) and maintained through the fifth reuse (R5).

Statistical comparisons show that R0 values across all three leaf concentrations differ significantly from those at the fifth reuse (R5). This suggests that tea leaf addition sustains high wort attenuation across reuse cycles, resulting in beers with consistent quality and profile.

The maintenance of yeast vitality during reuse may be linked to the high concentration of antioxidant compounds in *C. sinensis* leaves, such as catechins, flavonoids, and caffeine. This is particularly relevant for small- and medium-scale breweries, as it supports extended yeast reuse or maintains yeast health and beer quality, reducing off-flavor production caused by declining yeast vitality (Nishiyama et al., 2010; Wauters, 2021).

3.2 Viability

According to Table 4, no significant changes in yeast cell viability were observed across all treatments, meaning the number of viable yeast cells remained consistent regardless of leaf addition. Thus, tea leaves neither inhibited nor promoted yeast growth, supporting their potential use in brewing to maintain robust yeast vitality.

Similar findings were reported by Kordialik-Bogacka and Diowksz (2013), who found that yeast cell viability showed no statistical differences across ten generations, yet achieved a viability level above 98%, which is higher than the levels observed in this study. However, their study used wort fermentation at 10–15 °P at 9 °C. At the same time, the viability values here (below the ideal ≥ 95% for reuse) may be due to systematic or random errors, such as late sampling causing alcohol, hydrostatic, or pH-related stress on yeast cells.

Table 3. Attenuation percentage and yeast vitality.

			Attenuation percentage			
Reuse (%)	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**
0	44.06 ± 3.77^{Bbc}	44.44 ± 0.84^{b}	48.33 ± 1.33^{ab}	51.82 ± 0.74^{ab}	43.54 ± 0.90^{b}	56.21 ± 0.75^{Aa}
20	45.80 ± 1.61^{Bbc}	44.87 ± 1.40^{b}	46.13 ± 0.67^{b}	49.28 ± 0.77^{ab}	47.08 ± 1.24^{ab}	52.10 ± 0.32^{ABa}
40	39.76 ± 0.31^{Ce}	45.40 ± 0.32^{d}	51.72 ± 0.32^{abc}	53.54 ± 0.01^{ab}	47.42 ± 2.38^{cd}	54.31 ± 0.50^{Aa}
			Yeast vitality			
Reuse (%)	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**
0	0.7 ± 0.06^{a}	0.65 ± 0.06^{ab}	0.46 ± 0.06^{ab}	0.41 ± 0.06^{b}	0.52 ± 0.06^{ab}	0.10 ± 0.01^{c}
20	0.6 ± 0.06^{ab}	0.86 ± 0.06^{a}	0.23 ± 0.06^{c}	0.19 ± 0.06^{c}	0.40 ± 0.06^{bc}	0.14 ± 0.06^{c}
40	0.6 ± 0.06^{a}	0.18 ± 0.06^{bc}	0.14 ± 0.06^{bc}	0.41 ± 0.06^{ab}	0.39 ± 0.06^{abc}	0.12 ± 0.06^{c}

Statistical differences are indicated by different letters in the same column by Tukey's multiple comparison test at $p < .05$.

3.3 Antioxidant activity and phenolic compounds

Table 5 presents the antioxidant potential of the produced beers, as determined using the DPPH method and phenolic compounds.

Table 5 indicates that beer itself has inherent antioxidant potential, as seen in the 0% treatment (no leaf addition), reaching a concentration of 80.9% in the final reuse with consistent values across prior reuses. These compounds likely originate from hops and malts, which contain polyphenols with antioxidant activity.

Beers with *C. sinensis* leaf additions show higher antioxidant concentrations, but the difference between 20 and 40% leaf additions is minimal, suggesting that 40% addition may not provide significant additional benefits.

Comparing R0 and R5 across treatments reveals no statistically significant differences, although variations in DPPH levels across generations suggest potential direct or indirect effects on yeast vitality. It is important to highlight that these compounds originate from different sources depending on whether the leaves are present or not. Combining DPPH data with phenolic compounds reveals varying concentrations of total phenolic compounds across the three leaf concentrations.

Table 5 shows no significant statistical variation in phenolic compounds in beers produced without tea leaves, maintaining consistency across reuses. Beers with 20% leaf addition show a decline in phenolic concentration, with statistically equal values from R2 to R5, while those with 40% addition show no significant differences.

Variations in DPPH and total phenolic analyses may result from process-related factors, such as increased oxidant production, with antioxidants in the 40% treatment potentially stabilizing these oxidants.

3.4 pH

Table 6 shows the pH analysis results for the produced beers.

The pH plays a crucial role in beer quality, particularly in controlling the pH of the wort during fermentation. However, the final pH of the beer largely depends on the wort's buffering capacity, the initial wort pH, and yeast growth (Gibson et al., 2007). At the end of fermentation, most beers have a pH ranging from 4.1 to 4.5, with wheat-based beers typically having slightly lower values. The final pH of a beer is primarily influenced by the fermentation process, during which yeast releases H^+ protons into the medium (Bamforth, 2001). As observed in Table 6, with rare exceptions, the produced beers maintained pH values within the expected range.

For comparison, a study by Schuina et al. (2017) produced beers with 50 and 100% hop substitution using *C. sinensis*. The resulting pH values were 4.84 and 3.83, respectively. Notably, both treatments with green tea leaves in this study achieved a more suitable pH range compared to the reference cited.

3.5 European Brewery Convention

Table 7 shows color values in EBC units for the produced beers.

Table 4. Viability percentage.

Reuse (%)	R0	R1	R2	R3	R4	R5
0	55.11 ± 10.03^{a}	58.99 ± 2.52^{a}	60.67 ± 1.97^{a}	64.12 ± 2.82^{a}	70.16 ± 2.19^{a}	65.30 ± 2.33^{a}
20	57.91 ± 0.49^{a}	63.77 ± 4.13^{a}	66.21 ± 3.27^{a}	62.42 ± 3.72^{a}	67.72 ± 1.07^{a}	65.95 ± 1.33^{a}
40	53.89 ± 14.56^{a}	60.89 ± 3.36^{a}	63.70 ± 5.78^{a}	66.40 ± 3.42^{a}	63.95 ± 1.46^{a}	60.67 ± 5.30^{a}

Statistical differences are indicated by different letters in the same column by Tukey's multiple comparison test at $p < .05$.

Table 5. Antioxidant activity and phenolic compounds.

			DPPH			
Reuse (%)	**R0 (%)**	**R1 (%)**	**R2 (%)**	**R3 (%)**	**R4 (%)**	**R5 (%)**
0	79.58 ± 1.31^{Aa}	79.81 ± 2.53^{a}	75.10 ± 5.87^{ab}	63.76 ± 0.43^{b}	68.01 ± 0.60^{ab}	80.99 ± 3.70^{Aa}
20	86.72 ± 1.82^{Aa}	87.04 ± 0.87^{a}	88.58 ± 0.09^{a}	77.44 ± 1.92^{b}	81.99 ± 0.50^{ab}	82.03 ± 3.14^{Aab}
40	90.96 ± 1.40^{Aa}	86.99 ± 0.45^{ab}	89.65 ± 0.21^{a}	86.55 ± 0.27^{ab}	86.24 ± 0.92^{ab}	79.27 ± 4.04^{Ab}
			Phenolic compounds			
Reuse (%)	R0 (EAG/L)	R1 (EAG/L)	R2 (EAG/L)	R3 (EAG/L)	R4 (EAG/L)	R5 (EAG/L)
0	363.41 ± 4.47^{a}	354.65 ± 16.98^{a}	347.21 ± 9.16^{a}	338.29 ± 10.32^{a}	336.98 ± 3.10^{a}	329.77 ± 18.82^{a}
20	366.97 ± 11.34^{ab}	395.97 ± 9.93^{a}	353.02 ± 4.13^{bc}	310.01 ± 8.71^{c}	356.59 ± 1.75^{bd}	325.73 ± 6.60^{cd}
40%	373.88 ± 4.03^{a}	411.40 ± 14.29^{a}	371.47 ± 10.34^{a}	363.64 ± 10.54^{a}	371.16 ± 18.04^{a}	382.02 ± 6.19^{a}

DPPH: 2,2-diphenyl-1-picrylhydrazyl. Statistical differences are indicated by different letters in the same column by Tukey's multiple comparison test at $p < .05$.

Table 6. pH analysis.

Reuse (%)	R0	R1	R2	R3	R4	R5
0	3.99 ± 0.08^{d}	4.06 ± 0.02^{d}	4.18 ± 0.01^{cd}	4.45 ± 0.01^{b}	4.72 ± 0.07^{a}	4.35 ± 0.03^{bc}
20	3.89 ± 0.05^{b}	4.03 ± 0.02^{b}	4.35 ± 0.03^{ac}	4.45 ± 0.02^{a}	4.49 ± 0.04^{a}	4.22 ± 0.04^{c}
40	4.14 ± 0.20^{bc}	4.04 ± 0.01^{bc}	4.30 ± 0.03^{bd}	5.90 ± 0.01^{a}	4.62 ± 0.02^{d}	4.28 ± 0.03^{bd}

Statistical differences are indicated by different letters in the same column by Tukey's multiple comparison test at $p < .05$.

Table 7. Beer color by European Brewery Convention.

Reuse (%)	R0	R1	R2	R3	R4	R5
0	21.3 ± 0.9^{aA}	19.4 ± 0.9^{ab}	17.1 ± 1.9^{abc}	15.0 ± 0.7^{bcd}	12.6 ± 0.7^{cd}	11.7 ± 0.6^{dC}
20	17.9 ± 1.1^{aAB}	18.9 ± 1.9^{a}	18.9 ± 2.5^{a}	14.1 ± 1.7^{a}	12.0 ± 0.5^{a}	15.3 ± 1.4^{aBC}
40	19.5 ± 0.9^{aAB}	17.6 ± 0.9^{a}	17.6 ± 1.3^{ab}	14.0 ± 1.3^{abd}	10.5 ± 0.1^{cd}	15.8 ± 1.7^{abcBC}

Statistical differences are indicated by different letters in the same column by Tukey's multiple comparison test at $p < .05$.

Table 7 shows that the addition of tea leaves affected the beer color, but variations were also observed across production batches, even in beers without leaf addition. This may be due to the storage time of the malts, as they were all purchased from the same batch. Similarly, the tea leaves were sourced from a single batch to avoid harvest variations, and both ingredients were stored correctly and sealed. However, they probably lost some organoleptic properties over time. Additionally, random errors during wort production, such as higher extraction of certain sugars or other factors, may have contributed to these variations.

According to Beer Judge Certification Program (BJCP, 2023), summer ales have an EBC range of 15.7–27.6. Despite the observed differences, most beers in this study were within the established parameters. Deviations from the BJCP range were mainly observed in the later production batches, supporting the hypothesis of physicochemical losses in the grains used throughout the process.

Statistical analysis comparing R0 and R5, as shown in Table 7, indicates that beers with leaf additions at both concentrations (20 and 40%) exhibit similarities in R0 and R5. Additionally, in the fifth reuse (R5), beers with leaf additions were statistically similar to those without leaves, suggesting low interference of *C. sinensis* leaves on beer color, regardless of their presence or concentration.

4 CONCLUSIONS

In summary, the presence of *C. sinensis* leaves, used in green tea production, influenced the reuse of yeasts in the production of summer ale beers across five generations. As shown, the leaves contributed to high wort attenuation during reuse. Alternating use of these leaves at a 20% substitution rate could be considered, as it provided the best results and stability, achieving higher attenuation and medium acidification, particularly between R2 and R3. Future studies could investigate the impact of alternating leaf use on yeast vitality over more than 10 reuses.

Notably, *C. sinensis* is a widely consumed tea with a lower commercial cost than hops, containing antioxidant compounds that affect yeast vitality and beer quality over time. This opens possibilities for further research on its long-term impact on beer shelf life.

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