CAN "GRAVATÁ" CONTRIBUTE WITH PHENOLIC COMPOUNDS TO A FUNCTIONAL AND INNOVATIVE FRUIT BEER?

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ABSTRACT

This study aimed to assess the effects of adding "Gravatá" (*Bromelia antiacantha* Bertol) pulp, a nonconventional edible plant (NCEP) from Brazil, to a craft Fruit Beer. Two formulations with different Gravatá pulp amounts (83 and 165 g/L) were compared to a control without the fruit. Gravatá-containing beers had lower pH (4.15-4.22), higher Total Titratable Acidity (TTA) (29.58-44.64 g/mL), increased bitterness (11.27-13.22 IBU), and greater turbidity (59.81-99.56 EBC) compared to the control. Total phenolic compounds (TPC; mg GAE/L) were higher in Gravatá formulations (302.99-352.64) than in the control (257.68). All formulations contained mainly ferulic acid, catechin, and epicatechin, with syringic acid found only in Gravatá samples. The antioxidant capacity in Gravatá-containing beers showed no statistical difference compared to the control. In the DPPH assay, the values were 0.465, 0.386, and 0.415 mmol TEAC/L, and in the FRAP assay, the values were 0.416, 0.443, and 0.469 mmol TEAC/L for the control beer and the beers containing 83 and 165 g/L of Gravatá, respectively. Principal Component Analysis (PCA) showed significant differences in acidity, turbidity, and TPC, with the highest impact in the sample with the highest pulp amount. Our findings indicated the potential value of incorporating a local NCEP into a functional beverage.

Keywords: Unconventional food plant; Craft beer; Phenolic compounds; Natural antioxidants.

1 INTRODUCTION

The growing demand for diverse, innovative, and high-quality food and beverages has popularized the craft beer industry, with global beer production exceeding 1.78 billion hectoliters in 2020.[1] Fruit beers, especially light beers with fruit, have gained consumer popularity.[2] Incorporating fruits with bioactive properties in beer production is widely accepted. The development of an amber ale beer with goji berries resulted in a beer with pleasant sensory characteristics and high antioxidant capacity.[3] Functional beer was developed with quince, enhancing its phenolic content and fruity sensory descriptors.[4] Brazil's natural resources, especially non-conventional edible plants (NCEP), like Gravatá (*Bromelia antiacantha* Bertol), are underexplored.[5] Gravatá contains lipids, fibers, carotenoids, phenolic compounds, vitamin C, and minerals like calcium, magnesium, manganese, and potassium, showcasing antioxidant capacity.[6,7] This fruit's composition and health benefits have spurred interest in its use, particularly in beer production, benefiting small producers and family farming.

This pioneering study assessed the impact of Gravatá pulp on the bioactive profile of a craft Fruit Beer, namely, its phenolic composition and antioxidant capacity. Principal Component Analysis (PCA) differentiated formulations based on their physicochemical and chemical composition.

2 MATERIAL & METHODS

Gravatá fruit (Concórdia, SC, Brazil), malts (Maltes Agrária, Brazil), hops (Barth Haas, Germany) and *Saccharomyces cerevisiae* W34/70 (Fermentis®, Lesaffre, France) were obtained locally. Analytical or chromatographic-grade reagents were used (Sigma Aldrich, USA). Brewing was adapted from literature.[8] Fruit Beer batches were brewed using a microbrewery (Brewhome 10 L, Brazil). The mashing process included Pilsen malt, Munich I malt, and mineral water, with increasing temperatures and continuous wort recirculation. The temperature was raised to 78 °C for 10 min for mashing-out, followed by addition of mineral water to achieve a 10 L final wort volume, boiled for 1 hour. Hallertau Magnum hops were added during the process. The base style was Munich Helles. Gravatá pulp was extracted and homogenized, and then added in the last 3 minutes of boiling before the final hops addition. Three formulations were prepared: control (no Gravatá), and two with 83 and 165 g/L of Gravatá pulp. Post-boiling, the wort was filtered, cooled, and transferred to a fermenter. Yeast was added. Primary fermentation occurred at 12 °C ± 1 °C for 7-9 days, followed by maturation at 0 °C ± 1 °C for 10 days. Carbonation was done, then the beers were bottled and carbonated for 10 days.

All physical, physicochemical, and microbiological analyses were performed in triplicate using decarbonated samples.[9,10] TPCs were determined using the Folin-Ciocalteau method. The DPPH assay was conducted following the literature Brand-Williams et al. (1995), expressed as mmol of Trolox per liter. The FRAP assay followed Benzie and Strain (1996) with adaptations, expressed as mmol TEAC/L.[11,12,13,14] Phenolic compounds were profiled using LC-ESI-MS/MS with an Agilent 1290 series chromatography system and QTRAP 5500 mass spectrometer. Chromatographic separation used a VENUSIL C18 column with a specific gradient program.[15] Statistical analysis involved ANOVA and Tukey's test (5% significance) using Minitab® Statistical 21.0 software. PCA was conducted using Statistica® 13.0 software. Data were autoscaled before PCA. Formulations included control, Fruit Beer with 83 g/L, and 165 g/L of Gravatá pulp.

3 RESULTS & DISCUSSION

The addition of Gravatá pulp to beer formulations resulted in a decrease in pH and an increase in acidity compared to the control sample. The apparent extract, real extract, dry extract, and alcohol content did not vary significantly among the formulations. The alcohol content ranged from 4.01 to 4.11%, showing a slight increase after fruit addition. These values are below the recommended range of 4.7 to 5.4% for Munich Helles style category of beer according to BJCP (2021) guidelines.[16] Bitterness significantly increased with higher pulp concentrations. Turbidity also increased significantly with higher pulp concentrations. There were no significant differences in the color of beers, although the formulation with the highest pulp concentration showed the highest values, probably due to the presence of carotenoids, particularly β-carotene, which is predominant in citrus fruits such as Gravatá.[6] No lactic acid and acetic acid bacteria, or contaminating yeast microorganisms were detected in any of the samples.

The addition of Gravatá pulp influenced the phenolic content and composition, and the *in vitro* antioxidant capacity of the beers (Table 1). The sample with the highest pulp addition had the highest TPC value (352.64 mg GAE/L), which was 37% higher than the control beer (257.68 mg GAE/L) and 16% higher than the sample with the lower fruit concentration (302.99 mg GAE/L). The TPC varied throughout the brewing process, increasing after fruit addition, decreasing during fermentation, lightly increasing again after maturation, and finally decreasing in the final product. The final control beer showed the highest antioxidant capacity in the DPPH method, while the FRAP assay indicated an increasing antioxidant capacity with higher pulp concentration. The major phenolic compounds found in all formulations were ferulic acid, catechin, and epicatechin, with syringic acid detected only in the fruit-containing formulations. Similarly, the antioxidant capacity of beers can vary depending on the beer styles, fruit addition, and brewing process, as already reported in the literature for beers containing Cornelian cherry[17] and mango pulp.[18]

Table 1. Quantification of total phenolic compounds and assessment of *in vitro* antioxidant capacity using DPPH and FRAP methods in Control Beer and Fruit Beer formulations with 83 and 165 g/L of Gravatá pulp.

Sample	Control	83 g/L	165 g/L
Total phenolic compounds (mg GAE/L)	257.68 ± 21.57 ^{C}	302.99 ± 25.75 ^{B}	352.64 ± 16.31 ^{A}
Antioxidant capacity – DPPH (mmol TEAC/L)	0.465 ± 0.153 ^{A}	0.386 ± 0.177 ^{A}	0.415 ± 0.079 ^{A}
Antioxidant capacity – FRAP (mmol TEAC/L)	0.416 ± 0.041 ^{A}	0.443 ± 0.089 ^{A}	0.469 ± 0.089 ^{A}

Results expressed as mean ± standard deviation (n = 9). Equal superscripts on the same line, in capital letters, do not indicate a statistical difference between the samples (p > 0.05)

PCA is a pattern recognition technique used to assess similarities and trends among samples in a dataset. Applying PCA, the first two principal components together described 59.96% of the data total variability, providing discriminatory information about the samples. PC1 accumulated 40.76% of the data total variance, followed by 19.20%, described by PC2. The score and weight graphs for the first two principal components are shown in Fig. 1. Analyzing the graph of scores between PC1 versus PC2 (Fig. 1a and b), we observe the formation of two groups according to the studied parameters. The first group consisted mostly of all samples of the formulation with the highest quantity of pulp (dots in blue, Fig. 1b) and a replica of the sample with the lowest quantity of pulp (replicate 3 of the formulation 83, dots in green, Fig. 1b). The second group was formed by the control sample and two replicates of the sample with less pulp (replicates 1 and 2 of formulation 83).

The loadings graph (Fig. 1c) indicates that all the evaluated effects are variables that significantly influenced the separation of the groups observed in the score graph, confirming that, in PC1 with a positive weight, the pH of the beers was responsible for distinguishing the distinct groups. Color, bitterness, % alcohol, real extract, and FRAP were factors that made the third beer replica with the lowest pulp concentration more similar to all the replicas of the formulation with more pulp. The beer with the highest fruit concentration exhibited characteristics of acidity, turbidity, TPC, and apparent extract with weights justifying its differentiation from the control beer. Although other components could have been assessed, we believe that with almost 60% variability in experimental data, it is sufficient to observe the influence of the studied parameters and their role in characterizing the samples.

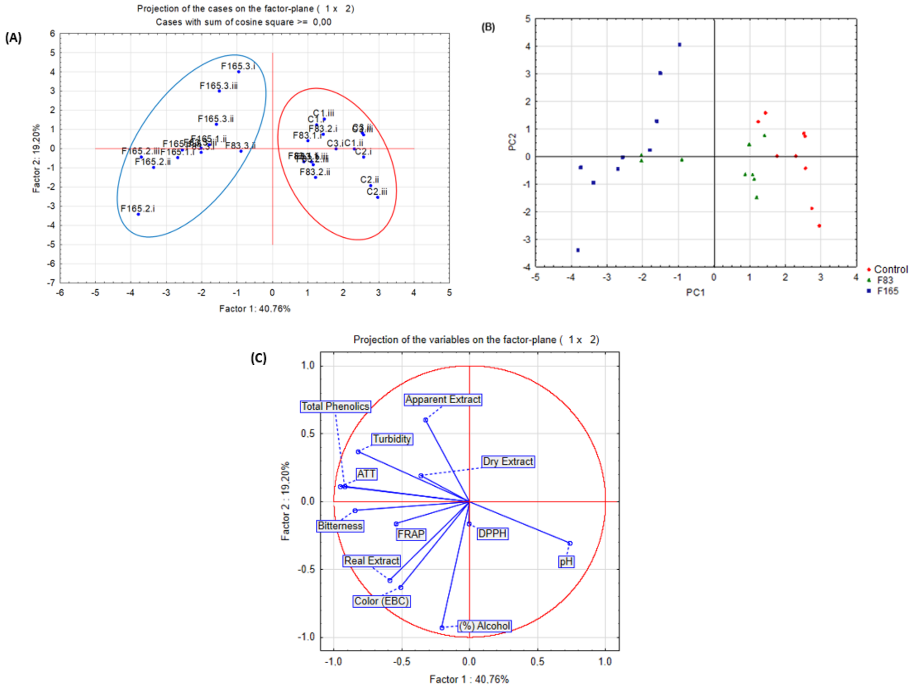

Figure 1 Key components in Control Beer (C) and Fruit Beer formulations with 83 (F83) and 165 g/L (F165) of Gravatá pulp (*n* = 9): a) similarity analysis results; b) visualization of similarity relationships through color mapping; and c) variable projection for evaluating analyses.

4 CONCLUSION

A unique craft Fruit Beer was successfully developed, featuring Gravatá pulp that significantly influenced pH, acidity, and bitterness, and added TPC and individual phenolic compounds to the samples. The FRAP assay revealed a slight increase in the antioxidant capacity of the beer due to the fruit pulp, although no statistical difference was observed among the samples. Notably, the formulations contained phenolic compounds such as ferulic acid, catechin, and epicatechin, with only the Gravatá-containing beers containing syringic acid, particularly in the higher pulp concentration. Utilizing Gravatá offers an opportunity to develop a unique and innovative beer with distinct functional characteristics.

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