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Production and Characterization of Craft Beers with Addition of *Campomanesia adamantium* O. Berg Fruits and Leave

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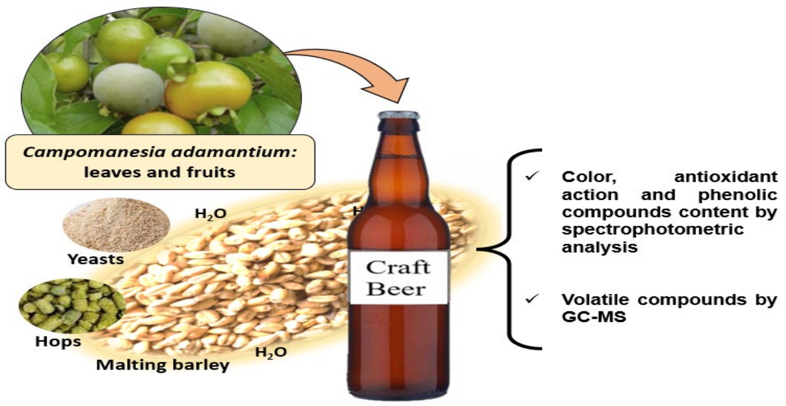
The increase in the consumption of craft beer is drawing attention to its characteristics. The search for compounds or products that increase stability and guarantee the quality of the beer is fundamental. Here is presented the preparation of two craft beers with the addition of aqueous extract of leaves or fruits of *Campomanesia adamantium* since this species is known for having a high antioxidant activity. Volatiles compounds and phenolic content, pH, colour and antioxidant action were determined for the samples. The results show that the alcohol content and the number of volatile compounds are on average for most beers. The sample prepared with *C. adamantium* fruits has a higher content of phenolic compounds and antioxidant potential, compared to the sample added with the aqueous extract of the leaves. Fruits of *C. adamantium* increased the antioxidant action of craft beers by 79.1%.

Graphical abstract



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1. Introduction

The Brazilian craft beer market grows every year and has become a more solid segment with products aimed at more demanding consumers [1]. Some breweries manufacture a single product, others already have a portfolio with a variety of beers, but both seek their styles with different flavours and aromas and with modifications and innovations in the production processes [2]. It is an alcoholic beverage made from a recipe that contains water, malt, hop and yeast, and combinations of different ingredients such as spices, herbs, fruits, among others, can be added in proportions that make it possible to obtain a novel drink with unique flavour and aroma [3, 4].

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To improve organoleptic characteristics, some craft breweries add starch adjuncts that are unmalted cereals in quantities that do not affect the progress of the process, such as corn, barley, sorghum in grain, rice, rye, among others [5]. The addition of these ingredients can result in different types and beer styles [6]. However, care must be taken with the addition and incorporation of raw materials into beer, as some substances can inhibit both the enzymatic activity during the hydrolysis process and interfere with the metabolic activity of the yeast during fermentation. It is well known that the main production steps are milling, maceration and fermentation and development under ideal conditions results in a product that is nutritionally rich in carbohydrates, amino acids, vitamins, minerals and phenolic compounds. So, the choice of yeast affects the chemical characteristics of beers [7, 8].

For this process, strains of *Saccharomyces cerevisiae* that are highly adapted to the conditions and substrate to be fermented are used. This microorganism is responsible for the production of several metabolites that influence the beer flavour and aroma, such as higher alcohols, glycerol, ethanol, among others [9]. Thus, in addition to paying attention to the process, it must be ensured that the fermentation conditions are as suitable as possible so that the active compounds present in the beer are within the correct limits so that there is no imbalance in the flavour, aroma and quality of the beer product [10].

Craft breweries seek alternatives to make the products stable without compromising the organoleptic characteristics, however, some factors can interfere with the quality and significant changes in flavour can occur, which is difficult to monitor [11]. Thus, some ingredients inhibit oxidation and improve the quality of the final product, such as fruits and spices; when added to the beer at the beginning of a certain stage of fermentation increase the content of bioactive substances in addition to enabling oxidative stability of the product [12].

Given the above, the addition of ingredients containing bioactive compounds can represent an alternative, as they can considerably contribute to both the quality of the beer and enhance the colour, flavour and influence the stability of the product [13]. Campomanesia adamantium (Cambess.) O. Berg is known as "guava", presents favourable characteristics to be added to beer. This plant has sweet fruits with a light acid content, known for presenting antioxidant properties, which has been associated with the presence of phenolic compounds [14].

Therefore, this work aimed to prepare craft beers with the addition of a new ingredient to enhance their functional characteristics. Two craft beers were produced with the addition of an aqueous extract of leaves and fruits of *C. adamantium.*

2. Results and Discussion

Three Pilsner-type craft beers were prepared, two with aqueous extracts of C. adamantium fruits or leaves and one that was used as a control. The antioxidant action (Wilks' lambda = 0.211; F = 2.134; p = 0.0013), TPC (Wilks' lambda = 0.302; F = 2.456; p = 0.0021), pH (Wilks' lambda = 0.212; F = 2.129; p = 0.0034) and volatile content (Wilks' lambda = 0.299; F =3137; p = 0.035) of the three craft beers showed significant differences.

The craft beers had EBC units of 14.01 ± 0.77 for the control and 14.05 ± 0.71 and 14.09 ± 0.64 for the craft beers prepared with fruits and leaves of C. adamantium, respectively (Table 1). According to Decree 9.902/2019, of Brazilian legislation, craft beers are called blonde beers (EBC < 20 units), which is the most consumed type of beer in Brazil [15, 16].

Table 1. Analysis of the technical parameters of craft beer with *C. adamantium.*

Parameters	Craft beers		
	CC	CAF	CAL
pH	4.51 ± 0.11	4.71 ± 0.08	4.60 ± 0.10
EBC units	14.01 ± 0.77	14.09 ± 0.64	14.05 ± 0.71
Total phenolic content (µg GAE mL^{-1} sample)	256.3 ±2.9	384.9 ±4.3	314.7 ±4.1
DPPH radical scavenging activity (% inhibition)	39.3 ± 0.1	70.2 ±0.7	54.7 ± 0.3

Control of craft beer (CC); Craft beer with addition of aqueous extract of *C. adamantium* fruits (CAF); craft beer with addition of aqueous extract of *C. adamantium* leaves (CAL).

The average pH value for a beer with an aqueous extract of *C. adamantium* leaves was 4.60 ± 0.10, and 4.71 ± 0.08 for an aqueous extract of *C. adamantium* fruits. The control presented, on average, a pH of 4.51 ± 0.11 (Table 1). The values presented by the craft beers in this study are in agreement with the results of Nardini and Garaguso [17]. Who analyzed some beers with the addition of fruit and observed a pH range between 3.60 and 4.87. Silva et al. [18] identified several flavonoid derivatives in *C. adamantium* fruits.

Craft beer with the addition of *C. adamantium* fruit extract had the highest content of phenolic compounds (384.9 ± 4.3 µg GAE mL^{-1}) followed by the one prepared with the leaves (314.3 ± 4.1 µg GAE mL^{-1}) and the control (256.3 ± 2.9 µg GAE mL^{-1}) (Table 1). Again, it is obvious that the addition of the specie is improving the properties of the beers. In a study by Pereira et al. [20], in which they produced craft beers with mixtures of cashew stalk and orange peel, the total phenolic content (516 to 727 mg GAE L^{-1}) was higher than those found in this study.

In the analysis of antioxidant action, craft beer with the extract of *C. adamantium* fruits presented an inhibition percentage of 70.2 ± 0.7, followed by a beer with the extract of the leaves (54.7 ± 0.3) and the control (39.3 ± 0.1) (Table 1). The addition of *C. adamantium* has contributed, in large part, to the antioxidant potential of craft beers. This is observed by the increase in 79.1% in the antioxidant potential of the craft beer prepared with the fruits. Compared to the control beer, and the increase in 39.2% for the craft beer prepared with leaves of *C. adamantium*. It confirms that the species has metabolites, mainly phenolic compounds, which add a better performance concerning the antioxidant action of the beverage. The control also showed some antioxidant activity, however, the samples which contained *C. adamantium* was much better. Some studies discuss the potential of using fruits and other ingredients as additives in beers in order to provide a functional beverage, a good example being the studies by Freire et al. [21], which added atemoia and sapodilla fruits to beers and it resulted in an increase in the antioxidant potential. Horincar et al. [22], prepared craft beers with eggplant peel extract and found a percentage of inhibition similar to that found in this study, about 78.

In the production of beer, although it seems a simple process, there is an interaction between variables mainly regarding the chemical and biochemical reactions involved in this process. Some compounds present in this beverage originate from the raw material and others are the result of biocatalysis performed by yeasts during fermentation [23]. Thus, in beer there are phenolic compounds represented by phenolic acids, flavonoids, tannins, among others, according to Zhao et al. [24]. Flavonoids are known to have antioxidant action, because of their chemical structure with electron acceptors and/or donor substitutes [19].

However, according to Martinez-Gomez et al. [23], the conduction of the manufacturing process can interfere with the content of these compounds and reduce their concentration in the final product, with the clarification or filtering step being the most critical, as this is where the loss of bioactives occurs. These compounds are of natural origin and their consumption brings health benefits, since their action has been proven, mainly in the control of cardiovascular systems, cholesterol metabolism, blood coagulation and glucose metabolism, in addition, as it is an antioxidant, antimelanogenic and anti-inflammatory [25,26]. According to Nardini and Garaguso [17], the nutritional quality of beer has a direct relationship with the alcohol content and antioxidant potential of beer. The analysis of alcohol content was between 5.11 and 5.69% (v v^{-1}) (Table 2). Bamforth [27] states that the average alcohol content of most beers is between 3 and 6% (v v^{-1}).

Table 2. Alcohol content and concentration of volatile metabolites of craft beers prepared with *C. adamantium* fruits and leaves.

Volatile Metabolites	Craft beers		
	CC	CAF	CAL
Ethanol (g L^{-1})	40.37±0.32	42.50±0.13	41.32±0.57
Acetic Acid (mg L^{-1})	35.77±0.84	34.25±0.93	34.11±1.27
Isoamyl alcohol (mg L^{-1})	1.01±0.02	1.22±0.03	1.13±0.04
Acetoin (mg L^{-1})	77.69±3.01	81.69±2.35	79.55±2.67
2,3-butanediol (mg L^{-1})	77.65±4.21	82.98±1.65	80.22±4.14
Phenylethyl alcohol (mg L^{-1})	67.19±1.45	70.92±3.28	69.12±2.64
Alcohol content (%)	5.11±0.09	5.69±0.12	5.23±0.11

Control of craft beer (CC); Craft beer with addition of aqueous extract of *C. adamantium* fruits (CAF); craft beer with addition of aqueous extract of *C. adamantium* leaves (CAL).

For homebrew to be consumed, some analyzes are required, going beyond measuring the alcohol content, it is also necessary to analyze the presence and quantification of volatile compounds, especially in the final product, to ensure its quality (Table 2). The samples had an acetic acid content between 34.11 ± 1.27 to 35.77 ± 0.84 mg L^{-1}. A result well below the one found by Pinu and Villas-Boas [28] which was 300.12 ± 20.66 mg L^{-1}. For these authors, acetic acid is the second most common and abundant volatile metabolite in fermented foods and beverages.

Isoamyl alcohol, acetoin, 2,3-butanediol and phenylylethyl were also quantified (Table 1). These compounds are abundant in fermented beverages and, as observed for the antioxidant action and the content of phenolic compounds, the craft beer produced with the addition of the aqueous extract of the fruits showed a higher amount of volatiles compared to the control. This result suggests that the incorporation of *C. adamantium* fruits or leaves as an additive possibly favored the presence of bioactive compounds, including antioxidants, which are necessary in this type of beverage, as they help in the quality of the beer, acting positively on its stability.

3. Material and Methods

3.1. Samples and preparation of plant extracts

The leaves and fruits of *C. adamantium* were collected in the Medicinal Plants Garden of Universidade Federal da Grande Dourados (UFGD). It was duly identified and deposited in the herbarium of the UFGD (#2192) and registered in the Brazilian genetic heritage control system (A055721). The leaves crushed in natura (3 - 5 mm) at 2% (m v^{-1}) were used to obtain the aqueous extract by a decoction process (98 ± 2°C for 10 min), afterwards it was kept at rest (about 30 min) for cooling, when it reached room temperature it was filtered, frozen and lyophilized (Cristo, Alpha 1-2 LD Plus). The fruits were crushed with a microprocessor at 25° C in the proportion 2% (m v^{-1}) and the mixture was promptly filtered, frozen and lyophilized. The extracts were obtained in triplicate and the yields (10.12 ± 0.72% for leaves and 25.45 ± 0.59% for fruits) were calculated using the mass of leaves or fresh fruits and the final extract.

3.2. Beer production

Beer was prepared as described by Piva et al. [29]. The wort obtained was poured into bioreactors, in which aqueous extracts of leaves (CAL) and fruits (CAF) of *C. adamantium* were added at a concentration of 0.1% (m v^{-1}) and kept at 20 °C. For the control, a bioreactor without the addition of extracts (control - CC) was used. Aliquots were collected for analysis.

3.3. Physicochemical analyzes (Color, pH, antioxidant action and total phenolic content)

The color analysis was performed using the Analytical Spectrophotometric method of the European Beer Convention – EBC 8.5 [30]. The samples were filtered on a paper filter (0.45 µm pore size) (A x 25 = color) and the absorption measured with a glass cuvette (10 mm) at 430 nm in a spectrometer (FEMTO 700 PLUS). The pH is analyzed with a phmeter (HACH PH31). The antioxidant action was measured by the DPPH radical method [31]. Percent inhibition was obtained based on absortions determined at 517 nm (FEMTO 700 PLUS). The total phenolic content was determined using the Folin-Ciocalteau method [32]. For this purpose, a standard curve was constructed with gallic acid (5 to 1000 µg mL^{-1}) and measured at an absorbance of 756 nm (FEMTO 700 PLUS). The result was expressed in µg of gallic acid equivalent (GAE) per mL^{-1} of samples. Analyzes were performed in triplicate.

3.4. Quantification of volatile compounds

Volatile compounds were quantified by GC-MS (GC-2010 Plus; GC-MS Ultra 2010, Shimadzu, Kyoto, Japan) as performed by de Pinu and Villas-Boas [28] and Piva et al. [29], the main ones being ethanol, acetic acid, isoamyl alcohol, acetoin, ranging from 0.1 to 10000 mg L^{-1}; 2,3-butanediol, phenylethyl alcohol in the range of 10 to 200 mg L^{-1}. Samples were analyzed in triplicate.

3.5. Data analysis

All data were evaluated by Factorial Analysis of Variance using the Statistic 13.3 software [33], $p < 0.05$ means that the values showed statistically significant differences.

4. Conclusions

Craft beers produced with the addition of aqueous extract of leaves or fruits of *C. adamantium* had parameters of color, pH and ethanol content similar to craft beers reported in the literature.

The addition of fruit extract to beer positively influenced both the antioxidant action and the content of phenolic compounds, indicating that the fruits of *C. adamantium* can improve the stability of beers and thus guarantee their quality.

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Author Contributions

Maria Helena Verdan: Data curation, Formal Analysis, Investigation, Visualization, Writing. Maria do Socorro Mascarenhas Santos: Data curation, Formal Analysis, Investigation, Visualization, Writing. Thiago Luis Aguayo de Castro: Data curation, formal analysis, investigation. Claudia Andrea Lima Cardoso: Conceptualization, Supervision, Resources, Project Administration, Writing.

References and Notes

[1] Silva, C. G. F.; Júnior, J. B. L. *Revista Científica e-Locução* **2021**, *1*, 20.

[2] Sanna, V.; Pretti, L. *Int. J. Food Sci*. **2015**, *50*, 700. [Crossref]

[3] Baiano, A. *Compr. Rev. Food Sci. Food Saf*. **2021**, *20*, 1829. [Crossref]

[4] Callejo, M. J.; Tesfaye, W.; González, M. C.; Morata, A. *IntechOpen* 2019, chapter 5 [Crossref]

[5] Cadenas, R.; Caballero, I.; Nimubona, D.; Blanco, C. A. *Foods* **2021**, *10*, 1726. [Crossref]

[6] Bettenhausen, H. M.; Barr, L.; Broeckling, C. D.; Chaparro, J. M.; Holbrook, C.; Sedin, D.; Heuberger, A. L. *Int. Food Res. J.* **2018**, *113*, 487. [Crossref]

[7] He, Y.; Dong, J.; Yin, H.; Zhao, Y.; Chen, R.; Wan, X.; Chen, P.; Hou, X.; Liu, J.; Chen, L. *J. Inst. Brew.* **2014**, *120*, 157. [Crossref]

[8] Pires, E. J.; Teixeira, J. A.; Brányik, T.; Vicente, A. A. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 1937. [Crossref]

[9] Zdaniewicz, M.; Satora, P.; Pater, A.; Bogacz, S. *Biomolecules* **2020**, *10*, 256. [Crossref]

[10] Baigts-Allende, D. K.; Pérez-Alva, A.; Ramírez-Rodrigues, M. A.; Palacios, A.; Ramírez-Rodrigues, M. M. *J. Food Compost. Anal*. **2021**, *100*, 103921. [Crossref]

[11] Lehnhardt, F.; Gastl, M.; Becker, T. *Crit. Rev. Food Sci. Nutr*. **2019**, *59*, 2642. [Crossref]

[12] Deng, Y.; Lim, J.; Nguyen, T. T. H.; Mok, I. K.; Piao, M.; Kim, D. *Food Sci. Biotechnol*. **2020**, *29*, 609. [Crossref]

[13] Deng, Y.; Lim, J.; Lee, G. H.; Nguyen, T. T. H.; Xiao, Y.; Piao, M.; Kim, D. *J. Microbiol. Biotechnol*. **2019**, *29*, 877. [Crossref]

[14] Coutinho, I. D.; Kataoka, V. M. F.; Honda, N. K.; Coelho, R. G.; Vieira, M. C.; Cardoso C. A. L. *Braz. J. Pharmacog*. **2010**, *20*, 322. [Crossref]

[15] Brazil. No. 9,902, of July 8, 2019. Amends the Annex to Decree No. 6,871, of June 4, 2009, which regulates Law No. 8,918, of July 14, 1994. [Link]

[16] Jardim, C. C.; Souza, D.; Machado, I. C. K.; Pinto, L. M. N.; Ramos, R. C. S.; Garavagia, *J. Beverages* **2018**, 4, 106. [Crossref]

[17] Nardini, M.; Garaguso, I. *Food chem.* **2020**, *305*, 125437. [Crossref]

[18] Silva, M. C. B.; Bogo, D.; Alexandrino, C. A. F.; Perdomo, R. T.; Figueiredo, P. O.; Prado, P. R.; Garcez, F. R.; Kadri, M. C. T.; Ximenes, T. V. N.; Guimarães, R. C. A.; Sarmento, U. C.; Macedo, M. L. R. *J. Med. Food*. **2018**, *21*,1024. [Crossref]

[19] Burda, S.; Oleszek, W. *J. Agric. Food Chem*. **2021**, *49*, 2774. [Crossref]

[20] Pereira, I. M. C.; Matos Neto, J. D.; Figueiredo, R. W.; Carvalho, J. D. G.; Figueiredo, E. A. T.; Menezes, N. V. S.; Gaban, S. V. F. *Food Sci. Technol*. **2020**, *40*, 749. [Crossref]

[21] Freire, B. R.; Lemes, D. S.; Moraes, A. S. S.; Gris, E. F.; Chaker, J. A.; Orsi, D. C. **2020**. *Rev. Agrarian* **2020**, *13*, 280. [Crossref]

[22] Horincar, G.; Enachi, E.; Bolea, C.; Râpeanu, G.; Aprodu, I. *Molecules* **2020**, *25*, 731. [Crossref]

[23] Martinez-Gomez, A.; Caballero, I.; Blanco, C. A. *Biomolecules* **2020**, *10*, 400. [Crossref]

[24] Zhao, H.; Chen, W.; Lu, J.; Zhao, M. *Food Chem.* **2010**, *119*, 1150. [Crossref]

[25] Zapata, P. J.; Martínez-Esplá, A.; Gironés-Vilaplana, A.; Santos-Lax, D.; Noguera-Artiaga, L.; Carbonell-Barrachina, Á. A. *LWT*. **2019**, *103*, 139. [Crossref]

[26] Osorio-Paz, I.; Brunauer, R.; Alavez, S. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 3492. [Crossref]

[27] Bamforth, C. W. *Nutr. Res*. **2020**, *22*, 227. [Crossref]

[28] Pinu, F.; Villas-Boas, S. G. *Metabolites* **2017**, *7*, 37. [Crossref]

[29] Piva, R. C.; Verdan, M. H.; Santos, M. S. M.; Batistote, M.; Cardoso, C. A. L. *J. Food Sci. Technol.* **2021**, *58*, 4403. [Crossref]

[30] Analytica, E. B. C. European Brewery Convention, Fachverlag Hans Carl Nürenberg Germany. Section, 2005, 4, 25.

[31] Kumaran, A.; Karunakaran, R. J. *Food Chem*. **2006**, *97*,109. [Crossref]

[32] Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. *Food Chem*. **2006**, *97*, 654. [Crossref]

[33] TIBCO Software Inc. Statistica (data analysis software system), version 13. **2017**. [Link]

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