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ORIGINAL ARTICLE

Brewing of craft beer enriched with freeze-dried cape gooseberry: a promising source of antioxidants

Fabricação de cerveja artesanal enriquecida com physalis liofilizada: uma fonte promissora de antioxidantes

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Abstract

The craft beer segment has been driving the beverage market due to the many different formulation possibilities. Among craft beers, fruity beers provide the consumer with flavors previously unknown. In this sense, cape gooseberry is a tropical fruit known for its unique flavor, whose production is seasonal, which makes it difficult to use throughout the year. One possibility of incorporating cape gooseberry into fruity beers is to subject the fruit to freeze-drying, ensuring a constant stock of pulp. The present study aimed to produce a craft beer added with freeze-dried cape gooseberry, to add mineral compounds, phenolic compounds, and antioxidant activity to the final beverage. Three beer formulations with different concentrations of freeze-dried cape gooseberry (20, 40, and 60 g/L) were produced and characterized. The results showed that the addition of the fruit directly influenced the physicochemical parameters pH, Total Titratable Acidity (TTA), Total Soluble Solids (TSS), alcoholic concentration, color, bitterness, real extract, primitive extract, and turbidity. In addition, the beers with freeze-dried cape gooseberry showed an increase in the content of some minerals, total phenolic compounds, and antioxidant activity. Thus, the addition of cape gooseberry is a good option to improve the functional characteristics of the beer, providing a differentiated product.

Keywords: *Physalis peruviana* L.; Physicochemical parameters; Total phenolic content; Antioxidant activity; Mineral composition; Fruity beer.

Resumo

O segmento de cervejas artesanais vem impulsionando o mercado de bebidas devido às diversas possibilidades de formulação. Entre as cervejas artesanais, as frutadas proporcionam ao consumidor sabores até então desconhecidos. Nesse sentido, a physalis é uma fruta tropical conhecida por seu sabor único, com produção sazonal, o que dificulta o uso durante todo o ano. Uma possibilidade de incorporar a physalis nas cervejas frutadas é submeter a fruta à liofilização, garantindo um estoque constante de polpa. O presente estudo teve como objetivo produzir uma cerveja artesanal enriquecida com physalis liofilizada, para adicionar compostos minerais e compostos fenólicos, e conferir atividade antioxidante à bebida final. Três formulações de cerveja com diferentes concentrações de physalis liofilizada (20, 40 e 60 g/L) foram produzidas e caracterizadas. Os resultados mostraram que a adição do fruto influenciou diretamente nos parâmetros físico-químicos, como pH, acidez total titulável, sólidos solúveis totais, concentração alcoólica, cor, amargor, extrato real, extrato primitivo e turbidez. Além disso, as cervejas com physalis liofilizada apresentaram aumento no teor de alguns minerais e compostos fenólicos totais, e na atividade antioxidante. Assim, a adição de physalis é uma boa opção para incrementar as características funcionais da cerveja, proporcionando um produto diferenciado.

Palavras-chave: *Physalis peruviana L.; Parâmetros físico-químicos; Teor de compostos fenólicos; Atividade antioxidante; Composição mineral; Cerveja frutada.*

Highlights

- Craft beer was enriched with freeze-dried cape gooseberry.
- Fruity beers showed higher functional characteristics compared to conventional beers.
- The addition of cape gooseberry to beers provided a differentiated product.

1 Introduction

Beer is one of the most consumed beverage in the world (Leão et al., 2018) with an annual global production of 1.96 billions hectoliters (Heredia-Sandoval et al., 2020). The brewing segment is between the oldest biochemical processes and is in full expansion, mainly characterized by the growth of craft breweries. The growth in the craft beer market is mainly driven by innovation, like the food industry as a whole (Gil & Tuberoso, 2021). Such, the relentless search for new sensory characteristics in craft beer has become a challenge for microbreweries in conquering new consumers. In the literature, there are some reports of the addition of fruit to beer, a process that gives rise to the so-called fruity beers.

The research reports an increase in the nutraceutical characteristics of the beer enriched with fruits. We can cite star fruit (Pal et al., 2021), red raspberries (Yin et al., 2021), cherry and plum puree (Fanari et al., 2020), hawthorn fruit (Gasiński et al., 2020), mango (Kawa-Rygielska et al., 2020), aronia berries (Jahn et al., 2020), omija (Deng et al., 2019), quince fruits (Zapata et al., 2019), okra pulp and powder (Xu et al., 2018), persimmon juice (Martínez et al., 2017), and goji berry (Ducruet et al., 2017).

Among the fruits, cape gooseberry (*Physalis peruviana* L.) is as health promoting food due to its levels of antioxidants and proteins. However, the fruit is perishable requiring a technique to preserving its original characteristics. In this sense, freeze-drying is an excellent alternative to increase the shelf life of fruits ensuring its availability during off-season periods, keeping the bioactive compounds (Bennemann et al., 2018). Considering that in the literature there is no mention of the incorporation of freeze-dried fruits in fruity beers, as well as there are no reports of enrichment of beers using cape gooseberry, the present study aimed to produce a craft beer with added freeze-dried cape gooseberry, and to characterize the beverage obtained based on in physicochemical parameters, total phenolic compounds, and antioxidant activity.

2 Materials and methods

2.1 Raw material preparation

Four kg of cape gooseberry 2020 crop (previously sanitized and frozen) were purchased at a specialized store in Itá – in the state of Santa Catarina (SC). Cape gooseberry was freeze-dried in a lyophilizer (Liobras model L 101, Brazil) for 96 hours. After the process, the fruit was crushed in a knife mill and stored until its use.

2.2 Craft beer production

In a mashing tub equipped with a false bottom, 16 L of mineral water (Puris, Brazil) were heated to 55 °C. Pilsen malt (3.2 kg) and wheat malt (1.3 kg) previously ground in a manual disk mill (Guzzo, Brazil) were added to the mashing tub. The mixture was left to stand at 55 °C for 10 min. After this time, gradual heating (1 °C/min) to 65°C was started, and the mixture rested for 60 min. The slow heating process was carried out again until reaching 75 ºC and kept at rest for 10 min. At the end of the mashing process, the iodine test was performed to confirm the saccharification of the starch.

The primary wort was separated from the malt bagasse using conventional filtration, under atmospheric pressure, with the filter cake itself (malt bagasse) being used as a filtering element. After filtering the primary wort, the cake was washed with 14 L of water (at 80 °C) to extract the residual sugar, obtaining the secondary wort. The mixture of both wort was boiled (±100 °C) at atmospheric pressure for 60 min. At 60 min of boiling, 8 g of Magnum hop pellets (Hallertau, Germany) were added, and at the final 5 min, 12 g of Perle hop pellets (Hallertau, Germany) were added. Then, the wort was cooled, present above the layer of precipitated proteins. The filtrate was divided into four equal aliquots, in fermenting containers, in which yeasts were added at the concentration indicated by the manufacturer. The fermenters were capped and kept at rest, fermenting at 18 ºC, for seven days.

After reaching 75% of the fermentation, aliquots of freeze-dried cape gooseberry were added to beer, in different concentrations: fermenter 1 (T1) 20 g/L, fermenter 2 (T2) 40 g/L, fermenter 3 (T3) 60 g/L, and fermenter 4 (Control) did not receive the addition of cape gooseberry, which was used as a control for later comparison between the other formulations. At the end of the seven days of fermentation, the beers were transferred to sealed containers and stored at 1°C for ten days. After the maturation process, the beers were bottled in previously sanitized containers. A sugar solution at a concentration of 6 g/L was added to each bottle to promote carbonation by the action of the remaining yeasts. The beers were stored for ten days at room temperature until the physicochemical analysis was carried out.

2.3 Physicochemical characterization

In natura cape gooseberry were characterized by pH, Total Titratable Acidity (TTA), and Total Soluble Solids (TSS). The four beer formulations T1, T2, T3 and Control were characterized by pH, TA, TSS, alcohol content, real extract, and primitive extract, all according to methodologies recognized and recommended by Instituto Adolfo Lutz (Instituto Adolfo Lutz, 2008).

The color (EBC) was determined by the spectrophotometric method at a wavelength of 430 nm (EBC - European Brewery Convention, 2005). Turbidity was determined by direct reading in a turbidimeter, and the value was expressed in Nephelometric Turbidity Unit (NTU). The bitterness expressed as the International Bitterness Unit (IBU) was determined after extraction by iso-octane (2,2,4-trimethylpentane) of the substances responsible for the hop bitterness, the iso-α-acids. For the determination of bitterness, 20 mL of each sample were acidified to 0.5 mL of 6.0 mol/L HCl and mixed with 20 mL of isooctane, followed by spectrophotometric measurement at a wavelength of 275 nm in a cuvette of 10 mm quartz (Philpott et al., 1997).

2.4 Mineral characterization

Freeze-dried cape gooseberry was dissolved in 5 mL of aqua regia (hydrochloric acid and nitric acid in a 3:1 ratio) and remained in an ultrasound bath at room temperature for 30 min. 1 mL aliquot of the extracted was diluted to 50 mL with Milli-Q water. The four beer samples T1, T2, T3 and Control were filtered and a 5mL aliquot was diluted to 50 mL in a 1% nitric acid solution. The analyzes were performed in an inductively coupled plasma optical emission spectrometry equipment (Thermo Scientific - ICAP 6500, United States) equipped with iTeVa Analyst software. The analytical curve, including the blank, was prepared with a 1% (v/v) HNO_3 solution in the concentration range of 0.010 - 2.0 mg/L.

2.5 Total phenolic content and antioxidant activity

For Total Phenolic Content (TPC) and antioxidant activity analysis the samples of freeze-dried cape gooseberry were firstly grounded in a mill. An aqueous extract (1:10 m/v) was prepared and taken in an ultrasonic bath for 15 min at 25°C. After this period, they were filtered and freeze-dried again.

The TPC was analyzed using the Folin-Ciocalteau reagent. An aliquot of 250 µL of the sample/extract was mixed with 250 µL of Folin-Ciocalteau reagent (diluted in distilled water, 1:1 v/v), 500 µL of saturated sodium carbonate solution, and 4 mL of distilled water. After 25 min at rest, the mixture was centrifuged for 10 min at 5000 rpm and the absorbance was read in a spectrophotometer (Analyser, 850 MI) at 725 nm. Gallic acid methanolic solutions with known concentrations in the range of 0 - 250 mg/L were used for calibration to plot the calibration curve. The results were expressed in mg gallic acid equivalent EGA per liter (mg EGA/L).

Antioxidant activity was determined by DPPH (stable free radical diphenylpicrylhydrazyl) and ABTS (2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] cation radical methods) assays. For the DPPH assay, beer and extract samples (25 µL) were added to 2 mL of 6.25×10^{-5} mol/L DPPH methanolic solution. The absorbance reading at a wavelength of 517 nm in a spectrophotometer (Analyser, 850 MI) was performed after the mixture remained at rest at room temperature for 30 min. Trolox methanolic solutions with known concentrations in the range of 0 – 2000 µmol/L were used to draw the calibration curve, and the results were expressed in µmolequivalent of Trolox (TE) per liter (TE/L).

For the ABTS assay, the radical was generated by the reaction of $ABTS^+$ (7 µmo/L) with 140 mM potassium persulfate and kept for 16 hours under protection from light at room temperature. The $ABTS^+$ solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. The absorbance reading was performed 6 minutes after adding 30 µL of beer sample and extract with 3 mL of $ABTS^+$ solution. Ethanol solutions of Trolox with known concentrations in the range of 0 – 2000 µmol/L were used to plot the calibration curve, and the results were expressed as µmol equivalent of Trolox/L.

2.6 Analytical determinations of volatile compounds

The volatile compounds (ethyl acetate, n-propanol, and isoamyl alcohol) were quantified using a Gas Chromatograph (GC), GC analysis, (Agilent, 7890A, MS/MS 7000), equipped with a VF-624ms chromatographic column (60 m × 250 µm × 1.4 µm) (Santa Clara, CA, USA). Injector and detector interface temperatures were 230 °C and 300 °C, respectively. The following oven temperature programming was used: start at 30 °C (4 min), raise to 60 °C at a rate of 5 °C/min, followed by heating to 186 °C at a rate of 18 °C/min. The carrier gas was nitrogen with a velocity of 32 cm/s. The sample preparation was done into 20 mL headspace vials and the injected volume was 200 µL in automatic splitless mode.

Acetaldehyde was quantified using High-Performance Liquid Chromatography (HPLC) (Prominence, Shimadzu) coupled to a diode array detector set at 365 nm. Chromatographic separation took place in an analytical column (Hypersyl C18). An isocratic method was performed by mobile phase 40% (v/v) of methanol and water.

2.7 Statistical analysis

All analyzes were performed in triplicate, with the results expressed as mean ± standard deviation. The results of the physicochemical analyzes, TPC and antioxidant activity were submitted to Analysis of Variance (ANOVA) and the means were compared by Tukey’s test with 5% significance.

3 Results and discussion

3.1 Cape gooseberry characterization

The maturation stage of fruits, *i.e.,* when them have reached the quality to be acceptable for consumers (Pinillos et al., 2011),can be assessed using different indexes, such as TSS,TTA, and pH. These parameters were evaluated, and the characterization results of *in natura* cape gooseberry are presented in Table 1.

Table 1. Characterization of *in natura* cape gooseberry.

Parameters	In natura fruit
pH	3.49 ± 0.02*
TA (g citric acid/100 g)	1.66 ± 0.01*
TSS (°Brix)	13.83 ± 0.15*

Mean ± standard deviation. *Means differ from each other Tukey's test ($p < 0.05$). TA: Titratable Acidity; TSS: Total Soluble Solids.

No specific Brazilian legislation regulates the classification and quality of *in natura* cape gooseberry fruits. In turn, the Colombian Technical Standard NTC 4580 (Instituto Colombiano de Normas Técnicas y Certificación, 1999), revised in 2022, establishes analytical standards to define seven stages of fruit maturation (denoted by seven colors). According to NTC, it is possible to categorize fruit maturation into stages 0 to 6, and as the set progresses, the classification number increases. The values of TSS, TTA, and Maturity Index (MI) are used to define the stage. The ratio of TSS/TA calculates the latter.

According to Table 1, *in natura* cape gooseberry is an acidic fruit (pH of 3.49). The TTA value of 1.66 g citric acid/100 g indicated a maturation near the last stage (color 6), and the TSS content of 13.83 º Brix was between that expected for the third and fourth stages, colors 2 and 3 respectively. The MI resulted in a value of 8.33 º Brix/g citric acid/100 g, which is expected between the last two maturation stages (colors 5 and 6) of *in natura* cape gooseberry. Thus, according to the Colombian Technical Standard NTC 4580, the fruits used as raw materials to produce artisanal beers were ripe.

The characterization of freeze-dried cape gooseberry is presented in Table 2.

Table 2. Characterization of freeze-dried cape gooseberry.

Analysis	Value
Co	0.003 ± 0.001[a] mg/g
Al	0.048 ± 0.045[a] mg/g
Na	0.484 ± 0.034[a] mg/g
Li	0.001 ± 0.000[a] mg/g
K	126.886 ± 1.949[a] mg/g
S	6.052 ± 0.221[a] mg/g
Zn	0.746 ± 0.554[a] mg/g
Mn	0.025 ± 0.019[a] mg/g
Ca	2.288 ± 0.133[a] mg/g
P	6.981 ± 0.614[a] mg/g
Mo	ND
Se	ND
Mg	5.359 ± 0.122[a] mg/g
Ni	0.001 ± 0.00 [a] mg/g
B	ND
Cr	0.007 ± 0.001[a] mg/g
Cu	0.076 ± 0.05[a] mg/g
Fe	0.159 ± 0.017[a] mg/g
TPC	47.37 ± 1.34* mg EAG/100 g
ABTS	158.60 ± 23.25* µmol TEAC/100 g
DPPH	133.32 ± 5.48* µmol TEAC/100 g
FRAP	588.40 ± 18.28* µmol Fe^{2+}/100 g

Mean ± standard deviation. ND: non-detected. Equal letters on the same line are not significant by the Tukey's mean test ($p < 0.05$). *Means differ from each other Tukey's test ($p < 0.05$).

From Table 2, the mineral composition of the freeze-dried cape gooseberry presented values close to those reported in the literature for the fresh fruit. According to (Pereda et al., 2019), the minerals contained in the fruits of this variety can contribute to human health, having beneficial effects on the circulatory, muscular, bone, and defensive systems.

The TPC for freeze-dried fruits was 47.37 GAE mg/100 g. The antioxidant activity was 133 µmol TE/100 g for DPPH and 158.60 µmol TE0 100/g for ABTS. These values are higher than those from literature for *in natura* fruits (Shenstone et al., 2020). Although, the comparison between the results from TPC and antioxidant activity of *in natura* and freeze-dried fruits is challenging. The drying process, itself, could interfere with the antioxidant compounds extracted by each method, make extracting of the antioxidant compounds harder or easier.

3.2 Fruity beers characterization

Table 3 presents the results of the physicochemical and analyzes of the produced fruity beers.

Table 3. Physicochemical characterization of produced fruity beers.

Parameters	Control	T1	T2	T3
pH	4.15 ± 0.01^{a}	3.93 ± 0.02^{b}	3.87 ± 0.01^{c}	3.81 ± 0.02^{d}
TA (%)	0.22 ± 0.01^{d}	0.35 ± 0.01^{c}	0.52 ± 0.01^{b}	0.73 ± 0.02^{a}
TSS (°Brix)	4.97 ± 0.21^{c}	5.03 ± 0.15^{bc}	5.43 ± 0.12^{b}	5.97 ± 0.15^{a}
Alcohol Content (%)	4.24 ± 0.04^{d}	5.13 ± 0.08^{c}	5.73 ± 0.15^{b}	6.13 ± 0.06^{a}
Color (EBC)	7.07 ± 0.05^{d}	11.76 ± 0.04^{c}	13.64 ± 0.08^{b}	21.46 ± 0.09^{a}
Bitterness (IBU)	11.62 ± 0.08^{a}	6.66 ± 0.28^{b}	10.23 ± 0.64^{a}	10.25 ± 0.83^{a}
Real Extract (%)	4.17 ± 0.03^{c}	4.23 ± 0.08^{c}	4.50 ± 0.03^{b}	5.26 ± 0.02^{a}
Primitive Extract (%)	10.86 ± 0.05^{d}	12.26 ± 0.10^{c}	13.44 ± 0.18^{b}	14.71 ± 0.09^{a}
Turbidity (NTU)	4.98 ± 1.30^{d}	42.37 ± 0.53^{c}	78.67 ± 1.15^{b}	103.67 ± 4.51^{a}

Mean ± standard deviation. Equal letters on the same line are not significant by the Tukey's mean test ($p < 0.05$). TA: Titratable Acidity; TSS: Total Soluble Solids.

From Table 3, it is possible to analyze how adding different concentrations of fruit to the beer influenced the parameters evaluated for the beverage obtained. It is observed that, with the increase in the amount of pulp, while the pH decreased, the parameters TTA, TSS, Alcohol Content, color, Real Extract, Primitive Extract, and Turbidity increased. Among these parameters, TTA, Alcohol Content, Color, Primitive Extract, and Turbidity showed significant differences between the measured values. In contrast, TSS, Bitterness, and Real Extract did not show a behavior proportional to adding fruit to beer.

pH and TTA values varied statistically to the control sample ($p < 0.05$). The acidity was proportional to the addition of acid in the extract, affecting the pH, that decreased. The presence of freeze-dried cape gooseberry in samples T1, T2, and T3 resulted in a greater extraction of organic acids, increasing the TTA values, and reducing the values of pH. All samples had a pH below 4.5, which has fundamental importance, because the reduced pH keeps the beer free of pathogenic microorganisms and prevents further contamination. For TSS values, samples T2 and T3 differed from the control sample. T1 showed no statistical difference in the TSS parameter when compared to T2 and control. These results are close to those reported for quince (Zapata et al., 2019), rice flakes and soursop pulp beers(Alves et al., 2020), and mango (Gasiński et al., 2020).

The addition of the freeze-dried fruit in the fermentation step, in which are produced esters and higher alcohols (Durga Prasad et al., 2022), slightly increased the alcohol content of the final beer. Alcohol production was proportional to adding sugars from the lyophilized extract to beer. When compared to control C (0 g), samples T1 (20 g), T2 (40 g), and T3 (60 g) produced 21, 35, and 45% more alcohol. The control sample was the one with the lowest concentration of alcohol content (4.24%), while samples T1, T2, and T3 had concentrations of 5.13, 5.73, and 6.13%, respectively. This variation can be attributed to the addition of freeze-dried cape gooseberry during the fermentation process, which contributed to fermentation due to the fermentable sugars present in the fruit. This demonstrates that freeze-dried cape gooseberry can be used as a source of fermentable sugars, partially replacing the amount of malt used. The increase in alcohol content in fruity beer was also reported after the addition of quince (Zapata et al., 2019), Cornelian cherry (Kawa-Rygielska et al., 2019), and grape (Veljovic et al., 2015).

According to the EBC color scale (EBC - European Brewery Convention, 2005), beers with values below 20 EBC are called light beers and values above 20 EBC are called dark beers. The color units (EBC) of the beers showed a progressive increase with the increase in the concentration of freeze-dried cape gooseberry in the beer, with the control (7.07EBC), T1 (11.76EBC), and T2 (13.64EBC) classified as light beer and T3 (21.46EBC) as dark beer. The variation in the color of the beers is associated with an increase in the concentration of coloring components dissolved from cape gooseberry. These components, like carotenoids, influence on the results of methods based on absorbance (Koren et al., 2020).

Bitterness, which is almost entirely derived from hop iso-α-acids, can change during beer fermentation and maturation due to the formation of other compounds responsible for flavor. As analyzed parameters, the bitterness of T1 beer showed a significant difference compared to the control, 6.66 and 11.62IBU, respectively. As T2 and T3, 10.23 and 10.25IBU respectively, they did not differ from the control. In relation to the real extract, which represents all solids present in the beer, such as sugars, proteins and dextrins, T1 (4.23%) did not show any significant difference compared to the control (4.17%), however T2 (4.50%) and T3 (5.26%) had values superiors. The values found in this study were higher than those reported by in the literature for beers added with cashew peduncle and orange peel (Pereira et al., 2020). As for the primitive extract values, all samples showed a significant difference.

The addition of freeze-dried fruit to beer positively influenced the turbidity values of the beverage, with all treatments significantly varying from the control. Turbidity ranged between 4.98 and 103.67 NTU, respectively for the control, T1, T2, and T3. The ingredients used in beer production are precursor sources of turbidity, which occurs from the polymerization reaction of phenolic compounds and association with some proteins. Furthermore, turbidity can be stimulated by factors such as the presence of oxygen and metal ions, pasteurization, and storage temperature. TSS in suspension can also contribute to increased turbidity in beer.

The mineral composition of fruit beers is presented in Table 4.

Table 4. Mineral content of beers enriched with freeze-dried cape gooseberry (mg/L).

Elements	Control	T1	T2	T3
Co	0.003 ± 0.001[a]	0.003 ± 0.001[a]	0.004 ± 0.001[a]	0.004 ± 0.001[a]
Al	0.070 ± 0.017[b]	0.053 ± 0.015[b]	0.480 ± 0.173[a]	0.000 ± 0.000[b]
Na	19.598 ± 0.175[b]	20.458 ± 0.342[a]	20.281 ± 0.183[a]	20.843 ± 0.036[a]
Li	0.003 ± 0.000[a]	0.004 ± 0.001[a]	0.003 ± 0.001[a]	0.004 ± 0.000[a]
K	388.687 ± 1.462[d]	701.633 ± 2.123[c]	1002.830 ± 0.139[b]	1154.300 ± 5.291[a]
S	63.123 ± 0.247[c]	63.870 ± 0.053[c]	71.680 ± 1.467[b]	106.633 ± 0.808[a]
Zn	0.004 ± 0.004[c]	0.026 ± 0.002[b]	0.013 ± 0.015[bc]	0.046 ± 0.001[a]
Mn	0.510 ± 0.010[a]	0.383 ± 0.015[c]	0.527 ± 0.021[a]	0.460 ± 0.001[b]
Ca	10.107 ± 0.247[d]	12.070 ± 0.072[c]	13.707 ± 0.076[b]	14.497 ± 0.178[a]
P	128.890 ± 0.455[c]	155.967 ± 0.55[a]	146.167 ± 3.121[b]	159.267 ± 0.153[a]
Mo	ND	ND	ND	ND
Se	1.372 ± 1.020[a]	0.234 ± 0.009[a]	0.210 ± 0.050[a]	0.258 ± 0.833[a]
Mg	77.790 ± 0.361[c]	84.960 ± 0.565[b]	89.490 ± 0.684[a]	89.333 ± 0.833[a]
Ni	ND	ND	ND	ND
B	ND	ND	ND	ND
Cr	0.005 ± 0.000[a]	0.007 ± 0.001[a]	0.005 ± 0.002[a]	0.006 ± 0.001[a]
Cu	0.093 ± 0.015[b]	0.087 ± 0.015[b]	0.127 ± 0.006[a]	0.024 ± 0.001[c]
Fe	0.067 ± 0.012[a]	0.037 ± 0.039[a]	0.117 ± 0.012[a]	0.086 ± 0.065[a]

Mean ± standard deviation. ND: non-detected. Equal letters on the same line are not significant by the Tukey's mean test ($p < 0.05$).

Among all the analyzed minerals, potassium had the highest concentration in all samples (388.687 to 1154.300 mg/L), followed by phosphorus (128.890 to 159.267 mg/L), sulfur (63.123 to 106.633 mg/L), and magnesium (77.790 to 89.490 mg/L). These results are in accordance with reports of the mineral content of 28 samples of Brazilian commercial beers (Tozetto et al., 2019) and top-fermented bee r (Zdaniewicz et al., 2021).

As shown in Table 2 and Table 4, among the analyzed minerals, only selenium was not found in the fresh and freeze-dried fruit, but all beer samples showed considerable levels. Selenium is a mineral that performs antioxidant functions and is essential as a trace element for human health and its deficiency is correlated with the occurrence of several diseases. The selenium levels present in beer come from barley malt and wheat malt used in beer production.

TPC and antioxidant activity of the four beer formulations are shown in Table 5. All samples added with cape gooseberry showed a significant difference compared to the control.

Table 5. Phenolic content, antioxidant activity, and volatile compounds of the beer formulations.

Parameters	Control	T1	T2	T3
TPC (GAE mg L^{-1})	216.62 ± 5.18^{d}	248.98 ± 8.70^{c}	297.89 ± 3.71^{b}	318.62 ± 6.58^{a}
ABTS (µmol TE L^{-1})	896.44 ± 90.76^{d}	1165.33 ± 94.52^{c}	1545.33 ± 61.10^{b}	1869.77 ± 111.82^{a}
DPPH (µmol TE L^{-1})	762.11 ± 18.36^{d}	853.22 ± 53.16^{c}	1041.00 ± 55.08^{b}	1202.11 ± 160.05^{a}
Ethyl acetate (mg L^{-1})	8.02	16.74	4.01	6.05
Isoamyl alcohol (mg L^{-1})	0.25	0.37	0.76	0.51
N-propanol (mg L^{-1})	12.60	12.91	25.28	9.47
Acetaldehyde (mg L^{-1})	4.50	8.10	1.04	1.30

Mean ± standard deviation. Equal letters on the same line are not significant by the Tukey mean test ($p < 0.05$). TPC: Total Phenolic Content.

The results in Table 5 indicate that the increase in TPC and antioxidant activity is directly dependent on the amount of cape gooseberry added to the beer. The absence of heat and the increase in the contact area of the freeze-dried fruit with the beer, as well as the breakage of the fruit's cell walls during drying, contributed to the release of phenolic compounds into the liquid (Shuen et al., 2021). These results may be related to the fruit antioxidants since its level are of the same order of magnitude as those found in beers added with the fruit. The beers enriched with freeze-dried cape gooseberry (T1, T2, and T3) presented high phenolic content, which contributes to antioxidant activity and therefore makes it a highly consumable food drink (Humia et al., 2020). Other interesting results is that the antioxidant activity of the beer obtained by the ABTS assay were higher than those obtained by the DPPH assay. Similar data have been reported in the literature and may be related to the different reaction kinetics between phenols and the ABTS radical cation and DPPH radical.

The values of TPC of beer samples were in the range of 216.62–318.62GAE mg/L. The highest and the lowest ABTS radical cation scavenging activities were found in control and T3 beers, respectively 896.44 and 1869.77 µmol TE/L, which was consistent with the result from DPPH radical scavenging activity assay, 762.11 and 1202.11 µmol TE/L. These results DPPH and ABTS assays are important to beer flavor stability during processing and storage and may have led to an increase in its nutritional value (Canonico et al., 2021). These are because greater DPPH assay could avoid beer staling due to the non-formation of saturated and unsaturated aldehydes because of oxidation. At the same time, the ABTS assay stabilizes active oxygen radicals and provides better flavor stability.

According to our data, the enrichment of craft beer with freeze-dried cape gooseberry during 75% of the fermentation step provided a considerable improvement in the nutritional quality of the beer, in terms of TPC and antioxidant activity, compared to control beer (without fruit addition). These increased amounts of chemical component in fruit beers might have beneficial effects on beer drinkers.

GC analysis (Table 5) indicated that the addition of freeze-dried cape gooseberry to beer had no specific influence in the final concentrations of evaluated fermentation by-products. The formation of aromatic compounds was not proportional to the fruit extract addition, which could indicate those compounds were more likely derived from other production steps slightly than additive cape gooseberry.

The ethyl acetate, responsible for fruits notes (Holt et al., 2019), ranged from 4.01 to 16.74 mg/Lin fruity beers. The isoamyl alcohol, responsible for banana flavor character (Martins et al., 2020), ranged from 0.25 to 0.76 mg/L. The n-propanol, which provides an alcoholic aroma to beer (Olaniran et al., 2017), ranged from

9.47 to 25.28 mg/L. In turn, acetaldehyde, which gives a green leaf and fruity flavor in beer, ranged from 1.04 to 8.10 mg/L.

4 Conclusions

In order to evaluate the incorporation capacity of phenolic compounds and antioxidant activity of freeze-dried cape gooseberry to beer, three different fruity craft beer formulations were evaluated. The results indicated that freeze-drying is a viable alternative for processing cape gooseberry. This technique, in addition to preserving the fruit, provides a beverage with higher mineral content, and concentrations of phenolic compounds and antioxidant activity. The addition of freeze-dried cape gooseberry to beer directly influenced its pH, TTA, TSS, alcoholic concentration, color, bitterness, real extract, primitive extract, and turbidity. Thus, the addition of cape gooseberry is an interesting option to improve the functional characteristics of beer, bringing innovation to the ever-expanding brewing sector.

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References

Alves, M. D. M., Rosa, S., Santos, P. P. A., Paz, M. F., Morato, P. N., & Fuzinatto, M. M. (2020). Artisanal beer production and evaluation adding rice flakes and soursop pulp (Annona muricata L.). *Food Science and Technology (Campinas)*, *40*(Suppl. 2), 545-549. http://dx.doi.org/10.1590/fst.36119

Bennemann, G. D., Botelho, R. V., Torres, Y. R., Camargo, L. A., Khalil, N. M., Oldoni, T. L. C., & Silva, D. H. (2018). Bioactive compounds and antiradical activity in grape pomace flours from different cultivars dehydrated in a freeze dryer and in an oven. *Brazilian Journal of Food Technology*, *21*, e2017205.

Canonico, L., Zannini, E., Ciani, M., & Comitini, F. (2021). Assessment of non-conventional yeasts with potential probiotic for protein-fortified craft beer production. *Lebensmittel-Wissenschaft + Technologie*, *145*, 111361. http://dx.doi.org/10.1016/j.lwt.2021.111361

Deng, Y., Lim, J., Nguyen, T. T. H., Mok, I. K., Piao, M., & Kim, D. (2019). Composition and biochemical properties of ale beer enriched with lignans from Schisandra chinensis Baillon (omija) fruits. *Food Science and Biotechnology*, *29*(5), 609-617. PMid:32419959. http://dx.doi.org/10.1007/s10068-019-00714-5

Ducruet, J., Rébénaque, P., Diserens, S., Kosińska-Cagnazzo, A., Héritier, I., & Andlauer, W. (2017). Amber ale beer enriched with goji berries – The effect on bioactive compound content and sensorial properties. *Food Chemistry*, *226*, 109-118. PMid:28254000. http://dx.doi.org/10.1016/j.foodchem.2017.01.047

Durga Prasad, C. G., Vidyalakshmi, R., Baskaran, N., & Tito Anand, M. (2022). Influence of Pichia myanmarensis in fermentation to produce quinoa based non-alcoholic beer with enhanced antioxidant activity. *Journal of Cereal Science*, *103*, 103390. http://dx.doi.org/10.1016/j.jcs.2021.103390

European Brewery Convention – EBC. (2005). *Analytica* (5th ed.). Nürnberg: Fachverlag Hans Carl.

Fanari, M., Forteschi, M., Sanna, M., Piu, P. P., Porcu, M. C., D’hallewin, G., Secchi, N., Zinellu, M., & Pretti, L. (2020). Pilot plant production of craft fruit beer using Ohmic-treated fruit puree. *Journal of Food Processing and Preservation*, *44*(2), 1-8. http://dx.doi.org/10.1111/jfpp.14339

Gasiński, A., Kawa-Rygielska, J., Szumny, A., Czubaszek, A., Gasior, J., & Pietrzak, W. (2020). Volatile compounds content, physicochemical parameters, and antioxidant activity of beers with addition of mango fruit (Mangifera Indica). *Molecules (Basel, Switzerland)*, *25*(13), 3033. PMid:32630803. http://dx.doi.org/10.3390/molecules25133033

Gil, K. A., & Tuberoso, C. I. G. (2021). Crucial challenges in the development of green extraction technologies to obtain antioxidant bioactive compounds from agro-industrial by-products. *Chemical and Biochemical Engineering Quarterly*, *35*(2), 105-138. http://dx.doi.org/10.15255/CABEQ.2021.1923

Heredia-Sandoval, N. G., Granados-Nevárez, M. del C., Calderón de la Barca, A. M., Vásquez-Lara, F., Malunga, L. N., Apea-Bah, F. B., Beta, T., & Islas-Rubio, A. R. (2020). Phenolic acids, antioxidant capacity, and estimated glycemic index of cookies added with brewer’s spent grain. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *75*(1), 41-47. PMid:31788720. http://dx.doi.org/10.1007/s11130-019-00783-1

Holt, S., Miks, M. H., De Carvalho, B. T., Foulquié-Moreno, M. R., & Thevelein, J. M. (2019). The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *FEMS Microbiology Reviews*, *43*(3), 193-222. PMid:30445501. http://dx.doi.org/10.1093/femsre/fuy041

Humia, B. V., Santos, K. S., Schneider, J. K., Leal, I. L., Abreu Barreto, G., Batista, T., Machado, B. A. S., Druzian, J. I., Krause, L. C., Costa Mendonça, M., & Padilha, F. F. (2020). Physicochemical and sensory profile of Beauregard sweet potato beer. *Food Chemistry*, *312*, 126087. PMid:31911355. http://dx.doi.org/10.1016/j.foodchem.2019.126087

Instituto Adolfo Lutz. (2008). *Normas analíticas do Instituto Adolfo Lutz: métodos químicos e físicos para análise de alimentos.* (4th ed.). São Paulo: Instituto Adolfo Lutz.

Instituto Colombiano de Normas Técnicas y Certificación – ICONTEC. (1999). *NTC 4580: 1999: frutas frescas: u chuva: especificaciones*. Bogotá: ICONTEC.

Jahn, A., Kim, J., Bashir, K. M. I., & Cho, M. G. (2020). Antioxidant content of aronia infused beer. *Fermentation*, *6*(3), 1-9. http://dx.doi.org/10.3390/fermentation6030071

Kawa-Rygielska, J., Adamenko, K., Kucharska, A. Z., Prorok, P., & Piórecki, N. (2019). Physicochemical and antioxidative properties of Cornelian cherry beer. *Food Chemistry*, *281*, 147-153. PMid:30658741. http://dx.doi.org/10.1016/j.foodchem.2018.12.093

Koren, D., Hegyesné Vecseri, B., Kun-Farkas, G., Urbin, Á., Nyitrai, Á., & Sipos, L. (2020). How to objectively determine the color of beer? *Journal of Food Science and Technology*, *57*(3), 1183-1189. PMid:32123439. http://dx.doi.org/10.1007/s13197-020-04237-4

Leão, P. R. P., Medina, A. L., Vieira, M. A., & Ribeiro, A. S. (2018). Beer sample decomposition using a reflux system for the monoelementar determination by F AAS/AES and multielementar determination by MIP OES. *Brazilian Journal of Food Technology*, *21*, e2017062. http://dx.doi.org/10.1590/1981-6723.6217

Martínez, A., Vegara, S., Martí, N., Valero, M., & Saura, D. (2017). Physicochemical characterization of special persimmon fruit beers using bohemian pilsner malt as a base. *Journal of the Institute of Brewing*, *123*(3), 319-327. http://dx.doi.org/10.1002/jib.434

Martins, C., Brandão, T., Almeida, A., & Rocha, S. M. (2020). Enlarging knowledge on lager beer volatile metabolites using multidimensional gas chromatography. *Foods*, *9*(9), 1-22. PMid:32932861. http://dx.doi.org/10.3390/foods9091276

Olaniran, A. O., Hiralal, L., Mokoena, M. P., & Pillay, B. (2017). Flavour-active volatile compounds in beer: Production, regulation and control. *Journal of the Institute of Brewing*, *123*(1), 13-23. http://dx.doi.org/10.1002/jib.389

Pal, H., Kaur, R., Kumar, P., Nehra, M., Rawat, K., Grover, N., Tokusoglu, O., Sarao, L. K., Kaur, S., Malik, T., Singh, A., & Swami, R. (2021). Process parameter optimization for development of beer: Star fruit fortified approach. *Journal of Food Processing and Preservation*, *46*(10), e15838.

Pereda, M. S. B., Nazareno, M. A., & Viturro, C. I. (2019). Nutritional and antioxidant properties of physalis peruviana L. Fruits from the Argentinean Northern Andean Region. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *74*(1), 68-75. PMid:30471071. http://dx.doi.org/10.1007/s11130-018-0702-1

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. (2020). Physicochemical characterization, antioxidant activity, and sensory analysis of beers brewed with cashew peduncle (Anacardium occidentale) and orange peel (Citrus sinensis). *Food Science and Technology (Campinas)*, *40*(3), 749-755. http://dx.doi.org/10.1590/fst.17319

Philpott, J., Taylor, D. M., & Williams, D. R. (1997). Critical assessment of factors affecting the accuracy of the iob bitterness method. *Journal of the American Society of Brewing Chemists*, *55*(3), 103-106. http://dx.doi.org/10.1094/ASBCJ-55-0103

Pinillos, V., Hueso, J. J., Marcon Filho, J. L., & Cuevas, J. (2011). Changes in fruit maturity indices along the harvest season in ‘Algerie’ loquat. *Scientia Horticulturae*, *129*(4), 769-776. http://dx.doi.org/10.1016/j.scienta.2011.05.039

Shenstone, E., Lippman, Z., & Van Eck, J. (2020). A review of nutritional properties and health benefits of Physalis species. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *75*(3), 316-325. PMid:32385801. http://dx.doi.org/10.1007/s11130-020-00821-3

Shuen, G. W., Yi, L. Y., Ying, T. S., Von Yu, G. C., Binti Yusof, Y. A., & Phing, P. L. (2021). Effects of drying methods on the physicochemical properties and antioxidant capacity of Kuini powder. *Brazilian Journal of Food Technology*, *24*, 1-14. http://dx.doi.org/10.1590/1981-6723.08620

Tozetto, L. M., Nascimento, R. F., Oliveira, M. H., Van Beik, J., & Canteri, M. H. G. (2019). Production and physicochemical characterization of craft beer with ginger (Zingiber officinale). *Food Science and Technology (Campinas)*, *39*(4), 962-970. http://dx.doi.org/10.1590/fst.16518

Veljovic, M., Despotovic, S., Stojanovic, M., Pecic, S., Vukosavljevic, P., Belovic, M., & Leskosek-Cukalovic, I. (2015). The fermentation kinetics and physicochemical properties of special beer with addition of Prokupac grape variety. *Chemical Industry & Chemical Engineering Quarterly*, *21*(3), 391-397. http://dx.doi.org/10.2298/CICEQ140415041V

Xu, K., Guo, M., Du, J., & Zhang, Z. (2018). Cloudy wheat beer enriched with okra [Abelmoschus esculentus (L.) moench]: effects on volatile compound and sensorial attributes. *International Journal of Food Properties*, *21*(1), 289-300. http://dx.doi.org/10.1080/10942912.2018.1454468

Yin, H., Deng, Y., Zhao, J., Zhang, L., Yu, J., & Deng, Y. (2021). Improving oxidative stability and sensory properties of ale beer by enrichment with dried red raspberries (Rubus idaeus L.). *Journal of the American Society of Brewing Chemists*, *79*(4), 370-377. http://dx.doi.org/10.1080/03610470.2020.1864801

Zapata, P. J., Martínez-Esplá, A., Gironés-Vilaplana, A., Santos-Lax, D., Noguera-Artiaga, L., & Carbonell-Barrachina, Á. A. (2019). Phenolic, volatile, and sensory profiles of beer enriched by macerating quince fruits. *Lebensmittel-Wissenschaft + Technologie*, *103*, 139-146. http://dx.doi.org/10.1016/j.lwt.2019.01.002

Zdaniewicz, M., Pater, A., Knapik, A., & Duliński, R. (2021). The effect of different oat (Avena sativa L) malt contents in a top-fermented beer recipe on the brewing process performance and product quality. *Journal of Cereal Science*, *101*, 103301. http://dx.doi.org/10.1016/j.jcs.2021.103301

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