

Publication status: Not informed by the submitting author

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<https://doi.org/10.1590/SciELOPreprints.9357>

Submitted on: 2024-07-10

Posted on: 2024-07-12 (version 1)

(YYYY-MM-DD)

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Abstract

Brazilian beer market presented remarkable increasement last years. Despite of this, Brazilian Amazon states presented discrete growth, with decrease in some important states as Amazonas. Cubiu (*Solanum sessiliflorum*) is a fruiting plant which has been used traditionally by people of the upper Amazon and Orinoco basin as artisanal medicine because of its bioactive molecules' richness. Because of world trend of innovative brewing products as fruit-beers, the aim of this work was to evaluate the composition and antioxidant activity of an artisanal beer added of cubiu. A total of 28 g/L of cubiu's pulp were added to the beer in maturation's phase, incubated at 4 °C for 10 days. Compared to a control assay, the resulting beer presented higher turbidity and soluble protein concentration. Total phenolic compounds concentration (435,93 µgGAE/mL) and antioxidant activity (66,36% of free radicals scavenged) were significantly greater than control assay ($p < 0,001$). The results indicates that a low amount of cubiu's pulp is sufficient to makes the beer functionally antioxidant. The subsequent efforts must to evaluate the acceptance of a beer added of *S. sessiliflorum* pulp to assessing its market potential.

Keywords: *Solanum sessiliflorum*, Artisanal beer, Antioxidant activity.

Cerveja artesanal adicionada de polpa de *Solanum sessiliflorum*: compostos fenólicos totais e atividade antioxidante

Resumo

O mercado brasileiro de cerveja apresentou notável crescimento nos últimos anos. Apesar disso, os estados amazônicos brasileiros apresentaram crescimento discreto, com queda em alguns estados importantes como o Amazonas. Cubiu (*Solanum sessiliflorum*) é uma planta frutífera que tem sido tradicionalmente utilizada pelos povos do alto Amazonas e da bacia do Orinoco como medicamento artesanal devido à sua riqueza em moléculas bioativas. Devido à tendência mundial de produtos cervejeiros inovadores, como as cervejas de frutas, o objetivo deste trabalho foi avaliar a composição e atividade antioxidante de uma cerveja artesanal adicionada de cubiu. Um total de 28 g/L de polpa de cubiu foram adicionados à cerveja em fase de maturação, incubada a 4 °C por 10 dias. Comparada a um ensaio controle, a cerveja resultante apresentou maior turbidez e concentração de proteína solúvel. A concentração total de compostos fenólicos (435,93 µgGAE/mL) e a atividade antioxidante (66,36% dos radicais livres neutralizados) foram significativamente superiores ao ensaio controle ($p < 0,001$). Os resultados indicam que uma pequena quantidade de polpa de cubiu é suficiente para tornar a cerveja funcionalmente antioxidante. Os esforços subsequentes devem avaliar a aceitação de uma cerveja adicionada de polpa de *S. sessiliflorum* para avaliar seu potencial de mercado.

Palavras chave: *Solanum sessiliflorum*, Cerveja Artesanal, Atividade Antioxidante.

1. Introduction

The current status of the Brazilian beer market is one of remarkable growth, as in number of manufacturers as in number of registered products. According to the “2021 beer yearbook”, the number of registered brewing products in Brazil increased of 8790 to 35741 between 2017 and 2021. Furthermore, Brazil has become a major exporter of beer, increasing of 80 to 241 tons of beer exported between 2011 and 2021 [1].

Despite of national growth trend, Brazilian legal Amazon presented a discrete growth in the same period, with decreasing as of registered products as of manufacturers in some states. The Amazonas state, the largest in the Brazilian Amazon, had the number of manufacturers reduced from 5 to 4 and growth of a mere 5% in the number of registered products, too much lower than the national average [1].

An interesting alternative to the development of innovative brewing products in Amazonia can be the use of native edible fruits. Amazonia is the largest rainforest of the world, containing 14003 described plants species [2], being among these 220 with edible fruits [3]. Some of these have flavors and aromas worldly famous as cupuaçu (*Theobroma grandifolium*) and açaí (*Euterpe oleraceae*). Others, besides flavor and aroma, present functional properties and are more known as traditional and artisanal medicine, as occurs to cubiu (*Solanum sessiliflorum*).

Considering that add amazonian fruits to brewing products development can result in beers, besides of presenting unique flavors and aromas, with functional properties, the aim of this work was to develop an artisanal beer added of fruits of cubiu (*Solanum sessiliflorum*), analyzing its composition and antioxidant activity compared to a control assay.

2. Material and Methods

2.1. Brewing protocol

The brewing wort was prepared using 1,0 kg of milled Pilsen barley malt (Best Malz®, Germany), 3,0 g of hops Nugget (alpha-acids 7%) and 2,0 g of Hallertau-Perle (alpha-acids 7%) for each 5,0 liters of potable water.

The mashing phase was performed using 55 °C for 15 minutes, 65 °C for 60 minutes and 75 °C for 15 minutes. The liquid was transferred to another vessel and boiled for 50 minutes, cooled at 20 °C and transferred to a new vessel for fermentation. The wort was fermented by *Saccharomyces cerevisiae* US-04 (Fermentis®, France) at 18 °C for 7 days.

For the maturation's phase, the fermented broth was divided in two vessels, 10 liters each one. In one of these, 280g of cubiu fruit were added to the beer. Both vessels were incubated at 4 °C for 10 days. The beers were carbonated in 600 mL amber glass bottles added of boiled sucrose (final concentration 6,0 g/L). In this phase, the bottles were incubated at 18 °C for 4 days and 4 °C for 6 days, when the beers were considered ready for consumption.

2.2. Response Variables

Density and Brix degree were analyzed, for the brewing wort and final product (beer), using a densimeter and a portable refractometer. Percentage of alcohol by volume (%ABV) was calculated according to previous description [4].

Total reducing sugar ([TRS], g/L) and total soluble protein ([TSP], g/L) concentrations were determined using DNS method and biuret method [5], respectively. Colors of the resulting beers were determined for European Brewing Convention scale (EBC), as previously described [4].

Total phenolic compounds was determined by Folin-Ciocalteu, expressed as µg of Gallic acid equivalent per ml of beer ([TPC], µgGAE/mL). Antioxidant activity was assessed by DPPH free radical scavenging assay, expressed in percentage of free radicals scavenged (%), both according to Sousa et al. [6].

2.3. Stastiscal analysis

For all response variables the assays were performed in triplicate and results were analyzed by Kruskal-Wallis test ($\alpha=0,05$) using JASP 0.14.1.0. [7].

3. Results

The resulting brewing wort presented original density (OG) of 1,041 g/mL (Brix degree 10,6%), while control and cubiu's beer presented final density (FG) in 1,007 ($\pm 0,001$) g/mL, without significant diference. Brix degree was different for both final products, being higher in control beer probably because of residual reducing sugar. Besides presenting more turbidity, Cubiu's beer presented significant higher concentration of soluble protein (table 1).

Add cubiu to the beer in maturation phase increased the concentration of total phenolic compounds in about 70%, when compared to the control beer. Free radicals were scavenged greater than 30% relative to control beer. Complete characteristics of each resulting beer are presented at table 1.

Table 1: Complete characterization of Cubiu and Control beers.

Variables	Beer with	Control beer	p-Value
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	cubiu		
[TRS] – (g/L)	ND	9,05 (±0,73)	N/A
[TSP] – (g/L)	14,53 (±0,56)	12,17 (±0,91)	0,019
%ABV	4,46 (±0,04)	4,46 (±0,05)	1,000
pH	4,29 (±0,01)	4,30 (±0,01)	1,000
Brix degree (%)	5,8	6,2	N/A
EBC Color Scale	25	18	<0,001
TPC (µgGAE/mL)	435,93 (±29,65)	259,26 (±15,33)	<0,001
%AA	66,36 (±1,36)	50,62 (±2,64)	<0,001

Subtitle: ND – Not Detected; N/A – Not Applicable.

4. Discussion

Final reducing sugar and soluble protein concentrations presented statistical significant difference, but it seems to be a technique artifact. There were not other characteristics, %ABV, or final density that corroborate these results as really significant. The only different response variable related to [TRS] and [TSP] was Brix degree, that occurred probably because of the higher [TRS] values.

Similar to the observed when using *Annona muricata* L. for artesanal beer [8], the final pH of the fruit-beer was not different when compared to the control assay. Furthermore, the Brix degree was similarly higher in the control assay, meaning a not significant contribution of sugars to the beer when adding cubiu in maturation phase.

The higher EBC color of the fruit beer is similar to the obtained by Ducruet et al. [9] when added goji berries to the beer in the maturation phase. These results indicate that addition of cubiu in the maturation phase of artisanal beer increases the turbidity of the beer, similarly feature of others artisanals fruit-beers.

Total phenolic compounds are produced by secondary metabolism of plants and may be classified as flavonoids, stilbenes, hydroxy-benzoic acids, hydroxycinnamic acids, hydrolysable tannins and proanthocyanidins. They come mainly, 70 %, from barley malt, but hops also contribute until 30% to this content. Most of TPC of the beer seems to be bioavailable, since the ingestion of 500 ml of beer [10].

TPC was significantly increased by addition of cubiu, similar to the observed when added goji berries [9]. Nardini and Garaguso [11] added 200 g/L of Apricots (*Prunus armeniaca* L.) and 300 g/L of Raspberries (*Rubus idaeus* L.), that resulted in TPC concentrations (454 and 465 µgGAE/mL, respectively) similar to the beer added of cubiu. Considering that we used 28 g/L of cubiu, these results indicates the high potential of this fruit to enrich the TPC concentration of beer when added in the maturation phase.

The enrichment of beer with fruits may add new flavors and aromas, besides increase the content of bioactive compounds and the oxidative stability of the Beverage. Usually, beer was

added of ascorbic acid to increase antioxidant activity and prevent the occurrence of off-flavors in the beer [12], but the addition of preservatives to the beers has created a rejection by the consumers, and this practice has been reduced by the manufacturers.

According to Assunção Júnior et al. [13], free-radical scavenging capacity lower than 65% is considered without antioxidant activity. By this way, the addition of cubiu in maturation phase turned a non antioxidant beer in an antioxidant beer, increasing the free-radical scavenging capacity from 50,62% to 66,36%. This result is similar to the addition of *Aronia* [14], increasing the antioxidant potential of the beer from about 5 to 80 µg/mL of ascorbic acid equivalent.

Despite of all biotechnological potential, cubiu's domestication and industrialization has been so slow, and its cultivation is eco-friendly performed mainly by small-owner farmers along all Orinoco and Amazon basin, since peruvian until brazilian Amazon [15]. The addition of small amounts of this fruit to beer can, besides resulting in an antioxidant beer, improve the economic activity along Amazon and increasing the incomes of farmers families.

5. Conclusions

The addition of *Solanum sessiliflorum* pulp to brewing process in maturation's phase results in a beer with higher turbidity and total phenolic compounds concentrations, when compared to control assay.

When compared to others fruits, remarkable lower amounts of *Solanum sessiliflorum* pulp are sufficient to makes the beer functionally antioxidant.

The subsequent efforts must to evaluate the acceptance of a beer added of *S. sessiliflorum* pulp to assessing its market potential.

Acknowledgements

Special thanks to Fundação de Amparo a Pesquisa do Estado do Amazonas (FAPEAM), Conselho Nacional para Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) because of financial support.

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Rita de Cássia Saraiva Nunomura: Chemical analysis, proofreading text.
Edson Júnior do Carmo: Financial support, proofreading text.
Spartaco Astolfi Filho: Financial support, proofreading text.

Conflicts of interest:

The authors declare not conflicts of interest.

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