

PHYSICOCHEMICAL AND SENSORY ANALYSIS OF CRAFT BEER MADE WITH SOURSOP (*ANNONA MURICATA* L.)

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ABSTRACT

Background. Owing to the scarcity of studies related to the use of soursop (*Annona muricata* L.) in the elaboration of craft beers, this study aimed to elaborate a soursop fruit beer using an artisanal method.

Materials and methods. To determine the most favorable moment for the inclusion of the fruit, the soursop pulp was added experimentally in three stages of the process: during the boiling of the wort (**C01**); on the 3rd day of fermentation (**C02**); during carbonation, with the use of soursop extract (**C03**). These treatments were submitted to a preliminary sensory evaluation and **C02** was considered the best beer with regards to taste, color, aroma, formation and persistence of the head and clarity. Thus, **C02** was evaluated with regards to its physicochemical, microbiological and sensory characteristics.

Results. The soursop fruit beer had the following characteristics: density of 1,012; pH 3.62; SSC 6.5°Brix, alcohol content 4.3 ABV; bitterness 39.3 IBU; and color 7.15 EBC. The centesimal analysis indicated the following amounts: ash 0.2 g/100 g, lipids 0.02 g/100 g, proteins 0.14 g/100 g and, among minerals, Ca and Na predominated in the beverage. The attributes evaluated in the sensory analysis showed an acceptability index greater than 70%, and 74% of the tasters indicated that they would probably buy the beer.

Conclusion. It was possible to produce a fruit beer with the addition of soursop pulp during fermentation which ensured the permanence of the sensory characteristics of the fruit.

Keywords: fermentation, alcoholic beverage, soursop

INTRODUCTION

Beer is defined as an alcoholic beverage obtained from the wort fermentation process made from malted cereal and grains (especially barley), yeast, water and hops (Albanese et al., 2018; Humia et al., 2019).

Currently, Brazil is the third largest producer of beer in the world, with a net production of 140 million hectolitres in 2017 and ranking only behind China and the United States (Marcusso and Müller, 2017).

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The growth of the beer market in Brazil is directly related to the emergence of craft breweries or micro-breweries, which between 2018 and 2019 grew in number by about 35%, reaching a total of 1,209 registered breweries (Müller and Marcusso, 2019).

With the growth of the craft beer market and increasingly more demanding consumers, a need has arisen to develop studies that investigate new inputs for brewing (Garavaglia and Swinnen, 2017; Gatrell et al., 2018). In this context, Aquilani et al. (2015) and Rodhouse and Carbonero (2019) affirm that the use of fruit in the preparation of beer can provide unique sensory attributes to the drink, with characteristic flavors and aromas, which can become a factor for consumer preference.

Despite the increasing number of craft breweries and the spread of “fruit beers” as a way to diversify and thus expand the beverage market, there are still few breweries that make technological use of soursop fruit (*Annona muricata* L.) in the elaboration of craft beers (Bicas et al., 2011; Virgen-Cecena et al., 2019).

Soursop comprises a group of fruit trees of economic importance in several countries, which are, in order of relevance, Mexico, Brazil, Venezuela and Costa Rica. In Brazil, the importance of commercial cultivation is due to the increased demand for tropical fruits, in addition to the possibility of use in other industrial sectors (São José et al., 2014).

Although the fruit possesses interesting nutritional and organoleptic characteristics, its high perishability is as one of the main obstacles to the marketing of the fruit in its fresh form, and for this reason many producers end up transforming it into pulp that is intended for use in various food products, such as juices, ice cream, compotes, jams and mousses (Chang et al., 2018; Quek et al., 2013; Quintana et al., 2018).

Therefore, the preparation of a beer based on soursop pulp can add value to the fruit, reduce post-harvest loss, provide producers with a guaranteed demand, and provide the market with a new biotechnological alternative. As such, the objective of this study was to elaborate a fruit beer using soursop pulp, evaluate its physicochemical and sensory characteristics and examine the technological possibilities of this fruit.

MATERIALS AND METHODS

Preparation of soursop pulp for experiments

Soursop pulp (*Annona muricata* L.) was purchased in the Municipal Market of the city of Manaus, Brazil. The packaging was washed with a neutral detergent and running water, and the contents were divided into portions of 500 g and kept in zip-lock plastic bags at -20°C until the beginning of the experiments.

Fruit beer formulations were prepared using the soursop pulp and added at different stages of the experimental production process, which were as follows: (C01) – 15% (v/v) of fresh soursop pulp was added in the last 15 minutes of the boiling of the mash; (C02) – 15% (v/v) of pasteurized soursop pulp in zip-lock plastic bags (immersed in water at 65°C for 30 minutes, followed by an ice bath) and added on the third day of fermentation of the wort; (C03) soursop extract (5 g/L) was added to beer for the carbonation. The extract was obtained from 900 g of soursop pulp and 950 mL of vodka (36% alcohol content), kept under maceration for 20 days and concentrated in a rotary evaporator (model TE-211, Tecnal) at 40°C . The beers were assessed by sommeliers from the Amazon School of Beer – EAC, who evaluated sensory characteristics such as aroma, color, taste, formation and persistence of foam.

Elaboration of the soursop fruit beer

In a motorized roller mill (Malt grinder with two 304 grade stainless rollers, Daddy® mill), 3.7 kg Pilsen BWS® and 0.3 kg Vienna Castle Malting® type malts were dry ground to the point of exposing the endosperm of their interior while keeping the husk intact. Then, 16 L of water at 70°C and 3.88 kg of grist were gradually added to the mash tun, followed by constant mixing. The temperature was adjusted to 68°C , which was strictly maintained for 60 minutes to allow the saccharification of starch in the fermentable sugars. The end of the process was verified with the 2% iodine test (v/v) (Curi et al., 2009) and, finally, the mash-out was performed by raising the temperature to 78°C for 15 minutes. In this step, the wort was clarified by gravity recirculation in the wort pot itself, with the aid of a filter (Bazooka) coupled to the valve of the pot, and the malt bagasse functioned as a filter mesh. This process was repeated from 8 to 15 times

until clarification of the wort, which was checked by visual identification.

The wort was transferred to the boiling pan, which also received the wash water from the bagasse. The bagasse was washed with 10 L of water at 78°C. Then the wort was heated to boiling. After 40 minutes of boiling, 15.4 g of Citra® hops T90 in pellet form (2019 harvest), with an alfa acid composition of 12.6% were added. After 50 minutes, the same amount of Tettnang® hops T90 in pellet form (2018 harvest), with an alfa acid composition of 2.27% were added. After 60 minutes, the process was completed, and the wort separation stage was performed. Subsequently, the wort was left to stand for 30 minutes for decanting of the trub.

The wort was cooled to a temperature of 29°C using an immersion chiller, then transferred to the fermenting vessel, where a volume of 14 L was confirmed, followed by the addition of 8.05 g yeast (Safale US-05®, Fermentis), which had been hydrated in 100 mL of mineral water (previously boiled for 5 minutes and cooled to room temperature). The fermentation of the wort was maintained at 19°C for 7 days, in an adapted refrigerator with a temperature regulator (model STC-1000, Shanghai Jingchuang Electronics Manufacturing Co., Ltd.). Subsequently, the beer was transferred to a maturator and kept at 0°C for 10 days.

At the end of maturation, the beer was filtered and poured into 600 mL bottles containing 12 mL of priming (inverted sugar). The bottles were closed with a pry-off cap and stored at 19°C for 10 days for carbonation and refermentation by the remaining yeasts, until the beer analysis began.

All equipment used during the process was properly sanitized with 70% alcohol and peracetic acid (PAC 200® ADPRO) prepared according to the manufacturer's instructions. All standards of good food handling practices were respected.

Physicochemical characterization of the wort

The pH determination was performed with a digital potentiometer (Quimis® model Q400RS). The determination of soluble solids content (SSC) was performed at 20°C using a portable refractometer and expressed in °Brix. Density was measured at 20°C using a conventional densimeter and expressed in g/mL.

Parameters controlled in fruit beer

The determination of color was based on the European Brewers Convention – EBC (EBC, 1987) standard, i.e., a 5 mL aliquot of decarbonated beer was centrifuged (5 minutes at 3,500 rpm) and the supernatant was transferred to a 10 mm cuvette. The reading was performed using a spectrophotometer (model SpectraMax® plus 384) at a wavelength of 430 nm. The result was calculated with the formula:

$$EBC = 25 \times d \times A_{430}$$

where:

- d – the dilution factor,
- A_{430} – absorbance at 430 nm.

According to the Beer Judge Certification Program (BJCP, 2016), the SRM (standard reference measurement) scale of the American Society of Brewing Chemists (ASBC) was also considered, according to Geisler and Weiß (2015) and calculated using the formula:

$$SRM = 12.7 \times d \times A_{430}$$

where:

- d – the dilution factor,
- A_{430} – absorbance at 430 nm.

The determination of the intensity of bitterness of the beer was based on Hall (1997) and estimated using the formula:

$$IBU = 0.7849 \times W_{oz} \times A\% \times U\% / V_{gal}$$

where:

- W_{oz} – hops in ounces (oz),
- $A\%$ – α -acid content as a percentage,
- $U\%$ – utilization percentage,
- V_{gal} – final quantity of beer in gallons, 0.7849.

The result was expressed in IBU (International Bitterness Units). The alcohol content was estimated using the methodology described by Brewer's Friend (2011), using the values of the initial density of the wort (OG – original gravity) and final gravity at the end of fermentation (FG – final gravity), and expressed in ABV (alcohol by volume), according to the following formula: %ABV = (OG – FG) × 131.25.

Centesimal chemical composition of the fruit beer and pulp

The moisture, protein, lipid and ash contents were determined according to the official methodologies of the Association Official Analytical Chemists (AOAC, 2005).

The humidity was obtained using the gravimetric method, with the sample kept at 105°C for 1 hour and weighed until it reached a constant weight. The ash content was determined by placing samples on a heating plate until a dark residue was formed and exposed to 550°C in a muffle furnace for 3 hours until it presented as a gray or white residue and showed a constant weight. The protein content was determined using the Kjeldahl method, using digestion by sulfuric acid at 350°C, distillation with sodium hydroxide (40%) and titration with sulfuric acid 0.05 M. The lipid content was evaluated using the method of direct extraction in a Soxhlet with petroleum ether as the extractor reagent, and the content was obtained by weighing after being in an oven at 105°C for 30 min. The total carbohydrate content was estimated by difference by subtracting the sum of lipids, proteins, ash and moisture from 100. Caloric value was determined using the Atwater conversion factors: 4 kcal/g (protein), 4 kcal/g (carbohydrates) and 9 kcal/g (lipids).

The determination of minerals was performed using an iCE 3000 series atomic mass absorption spectrometer (Thermo Scientific). Prior to measuring, the samples were digested using HNO₃ (nitric acid). The analyses were performed on the beer and the levels of calcium, magnesium, potassium, sodium, copper, lead, iron, manganese and zinc were quantified.

Coliform analysis

Analyses to determine total coliforms and thermotolerants were performed according to the method described by Hajdenwurcel (2004), and in accordance with Brazilian Ministry of Health ruling no. 12/2001, which defines microbiological standards for foodstuffs. The presumptive determination of total coliforms was performed in a series of five tubes containing lactose broth, which was incubated at 35°C for 24–48 hours. The samples that showed gas production were tested in brilliant green broth at 35°C for 24–48 hours and thermotolerant coliforms in *Escherichia coli* (EC) broth (Himedia, Mumbai-India) at 45°C for

24 hours. Samples that presented turbidity in the EC medium, with or without gas production, were considered confirmatory for *E. coli*, and NMP/mL values were calculated as described by Blodgett (2010).

Sensory analysis

Of the three fruit beer formulations, one was selected for an evaluation of sensory analysis that was performed in individual cabins that were free of noise, odors and with white light. The participation of volunteers was dependent on the signing of the informed commitment form (ICF). The project was approved by the Research Ethics Council (CEP) at the Universidade do Estado do Amazonas – UEA CAAE under approval N 12796319.4.0000.5016.

The beer was evaluated by 50 untrained tasters, which included students, technicians and professors of the Faculdade Estacio do Amazonas. These were selected according to inclusion and exclusion criteria and aged between 18 and 59 years. For each taster, a 40 mL sample of the beer was served in a disposable, transparent, acrylic glass at a temperature of 5°C ± 1°C. The tasters received a sensory analysis form for evaluation of the aroma, taste, color and general acceptance attributes, with a hedonic scale of nine points. For the intent-to-buy test, a five-point scale ranging from one (1 – definitely would buy) to five (5 – definitely would not buy) was used. The data obtained were used to calculate the acceptability index (AI), according to Dutcosky (2007).

Statistical analysis

The centesimal composition, excluding mineral analysis, was performed in triplicate, by which the means and standard deviation were calculated. The results from the sensory analysis were submitted to the analysis of variance (ANOVA) and Tukey test at the level of 5% significance for comparison of the means of the samples, using the program MINITAB®1.7.

RESULTS AND DISCUSSION

Choice of fruit beer formulation

The three fruit beers were evaluated by sommeliers of EAC who performed a descriptive sensory analysis covering the overall impression, visual aspects, aroma and flavor and palatability of the beers. Beer

C01 presented a light golden color, high turbidity, and a white head of medium formation, but low persistence. The aroma and soursop flavor had an excellent ratio and noticeable acidity, as well as sweetness and bitterness, which were balanced at a medium to low level. Beer **C02** presented a golden color, a white head of medium formation and low persistence and characteristics similar to **C01** in regard to the aroma and taste of soursop, though it did present low turbidity and an absence of sediment. On the other hand, **C03** showed a similar coloration to **C02**, though it did have higher turbidity. Head and carbonation were very low, and the aroma and flavor of the soursop were not in evidence, which, therefore, demonstrated the inefficiency of soursop pulp extract (as opposed to the pulp) in the formulation of fruit beer.

In beers **C02** and **C03**, it was observed that there was a darkening of the drink in relation to **C01**, which was possibly caused by the action of the polyphenol oxidase (PPO) and peroxidase (POD) enzymes. These enzymes are mainly responsible for the oxidative degradation of phenolic compounds and produce polymers of brown coloring (melanins) (Da Silva et al., 2009). The temperature used in pasteurization of the pulp may not have been efficient in the denaturation of these enzymes in **C02**, as was also the case for **C03**, whose extract was rotaevaporated at 40°C. According to Teixeira et al. (2006), temperatures above 80°C are required in order to reduce peroxide activity.

Although the beers **C01** and **C02** presented similar characteristics in the aromas and flavors of the soursop, we decided to select the **C02** for the later stages, due to the Brazilian preference for lighter and clearer beers (Coelho-Costa, 2015).

Course of fermentation

Figure 1 shows the physicochemical changes in the wort during seven days of fermentation. The initial density of the wort presented a reduction from the 2nd day of fermentation, however, on the 3rd day, after a sharp fall, it reached 1.015 g/mL. Finally, between the 5th and 6th days, it underwent a further reduction and stabilized at 1.012 g/mL. The monitoring of density is an essential parameter for estimating the alcohol content of the final product.

The initial (Fig. 1) pH of the wort (5.57) was considered to be within the limits, which ranged from 5.0

to 5.5 (Palmer, 1999) and, at the end of fermentation, the pH reached the value of 3.62, thus characterizing the final product as an acidic beverage. Similar results were obtained by Pantoja et al. (2005), who elaborated a fermented alcoholic beverage from soursop, for which the pH was 3.74. In research conducted by Alves et al. (2020), the pH ranged from 3.9 to 4.03 in the three beer formulations created using soursop pulp. The addition of soursop pulp can be a determining factor for the low pH of the beverage after the fermentation process, since the fresh soursop pulp has a pH of between 3.41 and 3.89 (Virgen-Cecena et al., 2019; Velasco-Hernández et al., 2020).

The beer wort presented a total Soluble Solid Content (SSC) of 12°Brix and 6.5°Brix at the end of fermentation (Fig. 1); a fact that is directly related to the consumption of wort sugars by yeast for carbon dioxide and ethanol production, which tends to decrease throughout the process (Kunze, 2006).

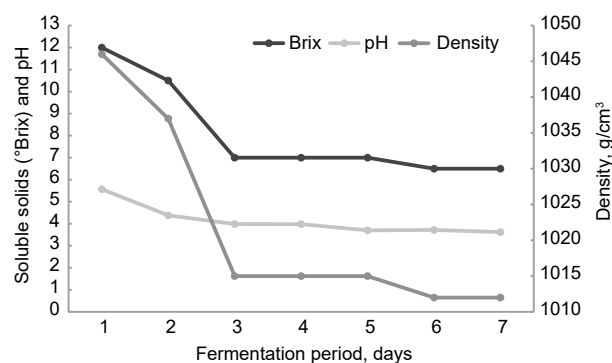


Fig. 1. Monitoring of the fermentation process of wort by analysis of total soluble solids, pH and density

The soursop fruit beer had an alcohol content of 4.4%. The EBC (1987) classifies the color of beer as light when there are less than 20 units of EBC, and dark when there are 20 or more units. The soursop fruit beer presented an EBC value of 7.15 and was therefore classified as “light” beer. Considering the Standard Reference Method (SRM) and color guide used by the beer styles guide (BJCP, 2016), the beer was classified as “yellow” with a value of 3.6.

Hops are the ingredient responsible, in part, for the aroma and bitterness of beer and, depending on

the formulation of the beer recipe, the greater the amount of hops and the boiling time in the wort, the greater the perception of bitterness of the drink (Nunes et al., 2017). The soursop fruit beer was rated with an IBU value of 39.

Centesimal and mineral composition

Despite the Brazilian Ministry of Health ruling no. 360/2003 not requiring nutritional labeling of fermented alcoholic beverages, the composition of macro and micro elements in fruit beer, as well as in the pulp of the soursop, was determined for evaluation of its nutritional quality.

In frozen fruit pulp, moisture is not considered to be an indicator of quality since it is a product with a high water content, which can end up being added to the pulp during processing or when harvesting is carried out during the rainy season (Brasil et al., 2016). The moisture (90.65 g/100 g) and ash (0.46 g/100 g) contents of soursop pulp were similar to those in the Food Composition Table (Universidade..., 2011): 89.2 g/100 g and 0.4 g/100 g, respectively. However, the lipid content was about 30% lower (Table 1). It is worth noting that these differences may be related to the cultivation of the soursop, degree of maturation, geographical origin and fertility of the soil, and the method of analysis employed (Rizzon et al., 2005).

The protein value of fruit pulp is commonly low and, in the pulp of the soursop analyzed, the protein content was 0.95 g/100 g, which was higher than the 0.7 g/100 g obtained by Teixeira et al. (2006). Regarding the carbohydrate content, Agu and Okolie (2017)

reported a level of 34.4 g/100 g in the fresh pulp of the soursop, which is greater than that found in the present study, probably due to the high moisture level of the frozen pulp used in our study.

It is known that the percentage of water in beer is around 95%, therefore ash does not exceed the decimal values. The levels of protein in this research were lower than those obtained by Abdoul-Latif et al. (2013), who assessed the composition of sorghum beer and found 0.65% protein. On the other hand, the same study did not find traces of lipids and found an even lower carbohydrate content (0.77 g/100 g) in their beer samples.

Sodium was the mineral that was found in the highest quantity in our soursop fruit beer, followed by calcium and magnesium (Table 2). The sodium content was higher when compared to Alcázar (2002), who analyzed beers of German origin and obtained 1.19 mg/L of this mineral. Sodium has no chemical or metabolic influence, but it is responsible for the softness and sweetness of beer and its recommended concentration is 75 to 150 mg/L (Pohl, 2008). In research carried out by Styburski et al. (2018) on beers from 11 countries, the authors concluded that the samples evaluated represented a good source of calcium, since the highest concentrations (0.31 g/L) were observed in those from Germany, while the beers from Portugal had the lowest concentration (0.05 g/L). These values are lower than those found in the present study (1.18 mg/L). Calcium plays an important role in the brewing of beers by acting in the pH reduction of

Table 1. Centesimal composition of soursop pulp and soursop fruit beer

Parameters	Soursop pulp	Soursop fruit beer
Moisture, g/100 g	90.65 ±1.29	94.99 ±0.06
Ash, g/100 g	046 ±0.06	0.2 ±0.04
Lipids, g/100 g	0.03 ±0.00	0.02 ±0.00
Protein, g/100 g	0.95 ±0.02	0.14 ±0.02
Total carbohydrates, g/100 g	7.9 ±0.98	4.6 ±0.04
Calories, Kcal/g	35.7 ±4.02	19.3 ±0.1

Table 2. Mineral composition of soursop fruit beer

Minerals, mg/L	
Calcium	1.18
Magnesium	1.13
Potassium	0.17
Sodium	1.3
Copper	<0.005
Lead	<0.005
Iron	<0.005
Manganese	<0.005
Zinc	<0.005

the wort, in the yeast metabolism and in the stabilization of enzymes involved in fermentation. However, it has little influence on the taste of the beer (Pohl, 2008).

The magnesium content was lower than the mean value of 126 mg/L obtained by Alexa et al. (2018), as were the potassium levels (412.5 mg/L) in the ginger craft beer produced by Martínez et al. (2017). High concentrations of magnesium contribute to the bitterness of beer, and its recommended concentration can be up to 30 mg/L (Pohl, 2008).

Copper, iron, lead, manganese and zinc were detectable with levels below 0.005 mg/L. These values are lower than those obtained by Tozetto et al. (2019), who found levels of zinc (0.04 mg/L), iron (0.06 mg/L) and copper (0.23 mg/L) in craft beer produced using ginger. In general, the mineral contents in soursop fruit beer were lower than those reported in the literature and it is believed that this is related to lower turbidity and the quantities of raw material (hops and malt) in its formulation (De Leão et al., 2018).

Coliform analysis

The analysis of the fruit beer indicated an absence of total coliforms and thermotolerants (<2 MPN/mL), as recommended by the Brazilian Ministry of Health ruling no. 12/2001. Thus, the results reflected the action of effective pasteurization of the fruit pulp, as well as compliance with the standards of good food handling practices, which guaranteed the microbiological quality of the product.

Sensory analysis

According to the questionnaire applied at the time of the sensory analysis, of the 50 tasters, 66% were female and 34% were male, and 76% were aged from

18 to 35 years. Around 56% of the tasters mentioned consuming beer frequently and, among these, 60.7% consumed the drink between 1 and 3 times a week. Regarding the consumption of soursop, 72% claimed to consume the fruit.

Table 3 shows the average scores given for the color and flavor attributes, in addition to the general acceptance, that varied between 7.0 and 8.0, which classified the beer between “liked reasonably” and “liked very much”, with the exception of the aroma attribute, which obtained a score of 6.9 “liked marginally” and “liked reasonably”. Although the aroma score was lower than the other evaluated attributes, it did not differ statistically between them.

According to the acceptability index (AI) of Dutcosky (2007), in order for a product to be evaluated with good impact in terms of its sensory properties, it must present an AI equal to or greater than 70%. Thus, when verifying the AI values for the soursop fruit beer, the beer’s attributes showed good acceptability, since all the attributes received a score equal to or greater than 76% (Table 3), most notably the general acceptance, which received 84.89% acceptability.

Regarding the intention to purchase, soursop fruit beer had a mean score of 3.98 ± 0.98 . Among the tasters, 40% indicated that they would probably buy the beer and 34% said they would definitely buy the beer. It is noteworthy that among these tasters, 40.5% are part of the group that does not consume beer frequently, demonstrating that beer pleased the taste of an audience that is not as accustomed to the drinking it. Only one taster expressed that they “definitely would not buy” the drink (Fig. 2).

Some tasters made comments when completing the sensory evaluation form, such as “very weak soursop

Table 3. Overall mean scores for each rated attribute and overall acceptance and acceptability index of the soursop fruit beer

Soursop fruit beer	Attributes			
	aroma	color	flavor	overall acceptance
Mean score	6.9 ± 1.80 ^a	7.5 ± 1.62 ^a	7.1 ± 1.92 ^a	7.6 ± 1.52 ^a
Acceptability index, %	76.67	82.89	78.44	84.89

Data presented as means ± standard deviation ($N = 50$). Values followed by the same letter did not differ statistically by the Tukey test at 5% ($p \leq 0.05$).

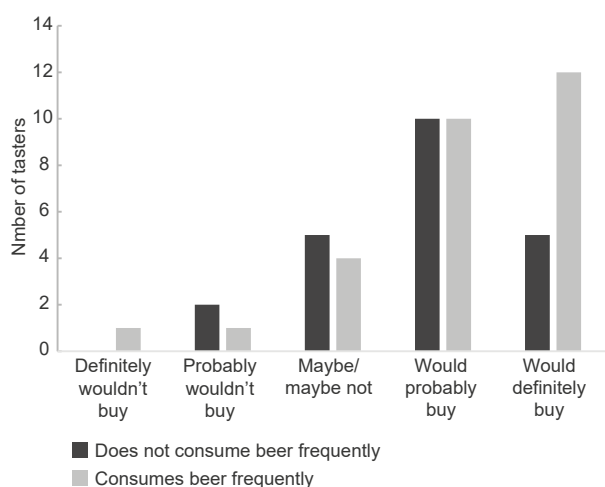


Fig. 2. Survey of purchase intention among beer-consuming and non-beer-consuming tasters

taste”, “very bitter beer”, “the color of the beer is very light” and “not much head”. It is worth noting that the volunteers used in the sensory analysis were untrained tasters or did not have the habit of consuming craft beers and such criticisms, when compared with the sensory evaluation report of the sommeliers of the EAC, presented divergences, namely: “evident soursop flavor and has excellent ratio”, “medium-low bitterness”, “golden color and mild turbidity” and “foam of good formation and low persistence”.

CONCLUSION

It was possible to produce a fruit beer with the addition of 15% v/v of soursop pulp on the third day of fermentation, which was the most promising treatment among the others performed. This ensured the permanence of the sensory characteristics of the fruit and produced a beer with a clear soursop flavor, as well as a balance of sweetness and bitterness. The soursop fruit beer presented physicochemical characteristics within the parameters expected for the base style. The evaluation and sensory analysis of the beverage demonstrated good acceptability and the pulp of the soursop presented itself as a good alternative for diversification of fruit beer styles, especially for breweries looking for innovation in their products.

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