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Cashew peduncle pulp in the production of Berliner Weisse craft beer: Sensory and physicochemical aspects

Adição de polpa de pedúnculo de caju na produção de cerveja artesanal Berliner Weisse: aspectos sensoriais e físico-químicos

Matheus Luz Alberti[1]; Vinícius Avanzi Barbosa Mascareli[2]; Diego Galvan[3]; Giselle Aparecida Nobre Costa[4]; Karla Bigetti Guergoletto[4]; Wilma Aparecida Spinosa[4]*

Highlights

Use of a co-product as an ingredient in a type of beverage.
Valorization of a pseudofruit and craft beer of brazilian origin.
Influence of different formulations on the characteristics of fruity beer.

Abstract

Among the extant beer types, Berliner Weisse is mainly characterized by its blend of barley and wheat malts, *Saccharomyces cerevisiae* for alcoholic fermentation, and *Lactobacillus* spp. for lactic fermentation. In this study, three formulations of Berliner Weisse were developed with various concentrations of barley, wheat malts, and hops. No variations were made in the concentrations of *S. cerevisiae, Lacticaseibacillus casei,* or cashew pulp. A *L. casei*-free formulation was used as a control. Physicochemical and sensory parameters were evaluated to characterize the formulations. The physicochemical data allowed for differentiation of the beverages in all evaluated parameters, except for the percentages of titratable acidity and diacetyl. From a sensory perspective, panelists classified the beer as acidic or fruity. The cashew peduncle pulp was proven to be a viable and attractive alternative for the production of Berliner Weisse-style beer with national characteristics and versatility in physicochemical and sensory parameters.

Key words: *Annarcadium occidentale* L. Beverages. Brazilian fruit. Fermentation. Tropical fruit.

[1] M.e in Food Science, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: matheus_luzalberti@hotmail.com
[2] Doctoral Student in Postgraduate Program in Food Science, UEL, Londrina, PR, Brazil. E-mail: vini.mascareli@gmail.com
[3] Prof. Dr., Department of Chemistry, Universidade Federal de Santa Catarina, UFSC, florianópolis, SC, Brazil. E-mail: diegogalvann@gmail.com
[4] Prof[as] Dr[as], Department of Food Science and Technology, UEL, Londrina, PR, Brazil. E-mail: gcnobre@uel.br; karla@uel.br; wilma.spinosa@uel.br
* Author for correspondence

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Resumo

Dentre os tipos de cerveja existentes, a cerveja Berliner Weisse tem como principal característica ser produzida a partir de um blend de maltes de cevada e trigo, *Saccharomyces cerevisiae* para a fermentação alcoólica e *Lactobacillus* spp. para a fermentação láctica. Neste estudo, foram desenvolvidas três formulações de Berliner Weisse com variações na concentração de maltes de cevada e trigo e lúpulo. Não houve variação nas concentrações de *S. cerevisiae, Lacticaseibacillus casei* e polpa de caju. Uma formulação isenta de *L. casei* foi formulada como controle. Foram avaliados parâmetros físico-químicos e sensoriais para caracterização das formulações. Os dados físico-químicos permitiram diferenciar as bebidas em todos os parametros avaliados, exceto quanto a porcentagem de acidez tilulável e diacetil. Do ponto de vista sensorial, os avaliadores classificaram as cervejas como ácidas e frutadas. A utilização da polpa do pedúnculo de caju se mostrou uma alternativa viável e atraente para a produção de cerveja estilo Berliner Weisse com características nacionais e versatilidade em seus parâmetros físico-químicos e sensoriais.

Palavras-chave: *Annarcadium occidentale* L. Bebidas. Fermentação. Frutas brasileiras. Fruta tropical.

Introduction

Beers are one of the most popular alcoholic beverages in the world and have been manufactured for millennia, since the nascency of human civilization. As the ingredients and technological improvements are being incorporated, consumption and production of beers has been increasing (Baiano, 2021; Villacreces et al., 2022).

Brewing requires primary raw materials such as water, malt, yeast, and hops (Baiano, 2021). However, craft beers have become increasingly common and are characterized by small, independently owned breweries that adhere to traditional brewing practices and ingredients to distinguish beer (Ducruet et al., 2017; Santos et al., 2021). During their manufacturing process, other components, such as spices and fruits, can be added. Such ingredients provide diverse flavors to beer, meeting the current market trends of consumers seeking new tastes and sensory experiences. In addition, these co-adjuncts can increase the bioactive compound content and oxidative stability, which can provide possible health benefits (Ducruet et al., 2017; Nunes et al., 2021).

Successful examples of these craft beers with adjuncts have already been reported, such as red ale beer with turmeric, pepper, and aroma hops (Nunes et al., 2021); amber ale beer enriched with goji berries (Ducruet et al., 2017); Pilsner beer with ginger (Tozetto et al., 2019); soursop (Santos et al., 2021); sage, dandelion, and nettle (Hayward et al., 2019); and wheat beer with cashew peduncle and orange peel (Pereira et al., 2020).

Cashew (*Anacardium occidentale* L.) is a potential co-adjunct that can be added during brewing. Cashew is a tropical pseudofruit native to Brazil with significant national economic importance, especially in the northern and northeastern regions, and is rich in vitamins C and B, tannins, minerals, and carbohydrates (Figueiredo et al., 2002; Pereira et al., 2020). Currently, the main cashew product is the processed nut, whereas other parts, such as the peduncle pulp, are mostly discarded. However, these co-products can be used in several food products, including juices, sweets, pulp, soft drinks, and fruity craft beer (Figueiredo et al., 2002; Pereira et al., 2020).

Berliner Weisse (BW) is a beer that can be potentially combined with cashew peduncle pulp. This beer has a low alcohol content (2%–3%) with high acidity and is usually mixed with sweet products to balance the taste. Its production employs mixed fermentation with alcoholic fermentation by Saccharomyces cerevisiae and lactic acid fermentation by *Lactobacillus brevis* or *Lacticaseibacillus casei* (Annemüller et al., 2008; Erbe & Brückner, 2000). Lactobacillus spp., also known as lactic acid bacteria (LAB), are popularly used in the production of various fermented foods. *Lactobacillus brevis* stands out in the brewing industry for having excellent growth at temperatures close to 30 °C and pH 4.6. The high incidence of LAB in beer can induce notable sensory and physicochemical changes in the beverage because of the super-attenuation effect exerted by the fermentation of dextrins and starch, which contributes to the quality of these beers (Dragone et al., 2008; Sakamoto & Konings, 2003).

From the perspective of appreciating a tropical fruit native to Brazil, this study aimed to develop and produce a fruity-flavored craft beer with mixed fermentation Berliner Weisse-type with variations in the concentrations of malts and hops, comparing them to a beer without the LAB addition. In addition, we evaluated the effects of lactic acid fermentation and formulations on the fruity characteristics of the beverage through sensory analysis and determination of physicochemical parameters.

Material and Methods

Raw materials

Barley, wheat malt, and Cascade aromatic hops (2018 harvest, 6.2% α-acid) were acquired from a local commerce (Londrina PR, Brazil). For alcoholic fermentation, *S. cerevisiae* yeast – US 05 Fermentis (Marcq-en-Baroeul, France) was used, and for lactic acid fermentation, a pure liquid culture of 9.30 log CFU mL^{-1} *L. casei* (Levteck Tecnologia Viva LTDA ME, Brazil). Pasteurized cashew peduncle pulp was acquired from Polpa Norte, Brazil, frozen, and stored at -18 °C until use.

Craft beer production

Three formulations of Berliner Weisse beer (BW2, BW3, and BW4) were prepared (Table 1). Formulation C1 (control) was not subjected to lactic acid fermentation, in which lactic acid was added after alcoholic fermentation during the maturation stage. Because of the simple and easily controllable experimental conditions, as well as the interest in comparing them, C1, BW2, BW3, and BW4 beer formulations were tested in a completely randomized design (CRD) with three replications of each treatment. All beers were produced using an automated Bravo Machine brewing (Bravo Brew, Brazil) with a production capacity of 20 L of beer wort.

Table 1
Formulations used to manufacture Berliner Weisse beer

Composition	Formulations			
	C1	BW2	BW3	BW4
Pilsen malt (kg)	1.5	1.0	1.5	2.0
Wheat malt (kg)	1.5	1.0	1.5	2.0
Hops (g)	6.5	6.0	6.5	8.0
Lacticaseibacillus casei (log CFU/mL)	0	9.3	9.3	9.3
Yeast (g)	5.0	5.0	5.0	5.0
Cashew peduncle pulp (mL)	100	100	100	100

Mashing

During the mashing step, a proportion of 4.0 L of H_2O per kilogram of malt was used. The water was filtered through an activated carbon filter to remove chlorine, and the pH was adjusted to 5.3 with 85% lactic acid. The blends of malts ground in a double roller mill were added to the sterilized brewing equipment, with the corresponding volume of water for each formulation (Table 1), at 45±1 °C following the temperature and time ramps: stages 1) enzyme activation (45 °C for 15 min), 2) protease (52 °C for 15 min), 3) saccharification – β-amylase (65 °C for 40 min), 4) saccharification – α-amylase (70 °C for 20 min), and 5) enzyme inactivation (78 °C for 5 min), according to (Kunze et al., 2014). All the mashing and filtration steps were performed simultaneously with automatic time and temperature control of the equipment.

Lactic acid fermentation

Formulated musts BW2, BW3, and BW4 were cooled outside the brewing equipment to 37±1 °C, and then *L. casei* was inoculated for the first stage of fermentation. The process was monitored through the kinetics of lactic acid fermentation by measuring the pH, lactic acid acidity, total soluble solids (TSS), and cell number every 4 h from the start of the process up to 24 and 28 h.

Boiling and hopping

After lactic acid fermentation, the formulations were transferred again to the sterilized brewing equipment, and boiled at 98 °C for 10 min. Thereafter, the Cascade hops were added and heated for 40 min and cooled using a water immersion chiller system to 25±1 °C.

Alcoholic fermentation

In this step, freeze-dried yeasts were added to the wort and transferred to a 12-L conical fermenter. The fermentation process was conducted out at a controlled temperature of 25±1 °C for 10 days until reaching an alcohol content between 3.5% and 4.0%, which was measured using a hydrometer.

Maturation and addition of cashew peduncle pulp

After alcoholic fermentation, the musts were stored at 0 °C for 12 days. The cashew peduncle pulp was pasteurized at 60 °C for 15 min, cooled, and added to fermented. In C1, lactic acid was added (based on previous tests; 10 mL L^{-1} beer) at the end of the maturation period.

Beer carbonation

Beer carbonation was forced until the pressure register in the stock keg showed 1.5 kgf cm^{-2}. The beer was filled against pressure in 600 mL sterilized glass bottles. The bottled beers were stores at 3±1 °C until analysis.

Analysis of cashew peduncle pulp and bottled beer

Physicochemical analyses of the cashew peduncle pulp included TSS, titratable acidity as a percentage of citric acid, and pH according to Association of Official Analytical Chemists [AOAC] (2012). In bottled beer, the color, turbidity, and diacetyl content were analyzed as described by the European Brewery Convention (Newell & MacFarlane, 1987), and the pH, alcohol content, and lactic acid titratable acidity were determined according to the AOCS (2012). In addition, microbiological analyses were performed on the bottled beers for total and thermotolerant coliforms, molds, and yeasts, as described by Zwietering et al. (2011).

Sensory analysis and ordering test

The panelists for the sensory test comprised 20 trained evaluators, adults over 18 and under 60 years of age, who were subjected to taste identification tests, odor recognition, and determination of the degree of differentiation, enabling them to identify and distinguish the evaluated attributes. The procedures were approved by the Research Ethics Committee of the State University of Londrina (ID: 89967518.7.0000.5231). Each beer sample (50 mL) was served at a temperature of 4 °C in transparent acrylic glasses coded with three-digit numbers for random evaluation. Each evaluator also received room temperature-drinking water to clean their palate before tasting each sample.

The ordering test was applied so that the samples were presented simultaneously, and the evaluators were asked to organize them in ascending order based on the intensity of the fruity aroma, sour taste, fruit flavor, and body attributes. The number of evaluations was higher than that suggested by the ABNT NBR ISO 8587:2015 standard, which requires 12–15 evaluators for ordering tests for product descriptions (International Organization for Standarization [ISO], 2015).

Statistical analyses

All statistical analyses were performed using the RStudio software. The physicochemical analyses were performed in triplicate, and their averages were derived; analysis of variance (ANOVA) and Tukey's test were performed to verify differences between the parameters of the beer samples (critical value: $p \leq 0.05$). For sensory analysis, the Friedman test (Newell & MacFarlane, 1987) was used to verify the differences between samples for each attribute, considering the number of samples was 4 and 20 evaluators at a 5% significance level.

Results and Discussion

Pulp physicochemical characterization

The physicochemical characterization of the cashew pulp was within the Brazilian legislative identity and quality standards (Ministério da Agricultura Pecuária e Abastecimento [MAPA], 1994). The pulp showed higher mean values of the analyzed parameters than the minimum values required by legislation. The pulp had a total acidity of 0.70 g 100 g^{-1}, pH of 4.0, and TSS of 10.86 °Brix at 20 °C, whereas the Brazilian legislation requires a minimum total acidity of 0.18, pH of 3.80, and TSS of 10 °Brix, respectively. Figueiredo et al. (2002) reported TSS values between 6.5 and 12.4 °Brix, total acidity between 0.2% and 0.3%, and pH between 4.3 and 4.9 (Figueiredo et al., 2002); whereas Pereira et al. (2020) reported TSS values of 11.2%, total acidity of 3.2%, and pH of 4.6 (Pereira et al., 2020). However, variations in physicochemical parameters can occur because of edaphoclimatic factors, such as rainfall and solar incidence, soil quality during harvest, or species variety. The good relationship between TSS and acidity shows that cashew peduncle pulp is a promising brewing co-adjunct, contributing to a new sensory experience and changes in the physicochemical parameters of Berliner Weisse craft beers.

Fermentation monitoring

The BW2 formulation had the lowest TSS content at the beginning of fermentation (Figure 1). Hence, LAB required a longer adaptation time, presenting a count of 16.06 log CFU mL^{-1} after 8 h of fermentation, whereas formulations BW3 and BW4 presented superior counts of 16.41 and 19.31 log CFU mL^{-1}, respectively, for the same time interval.

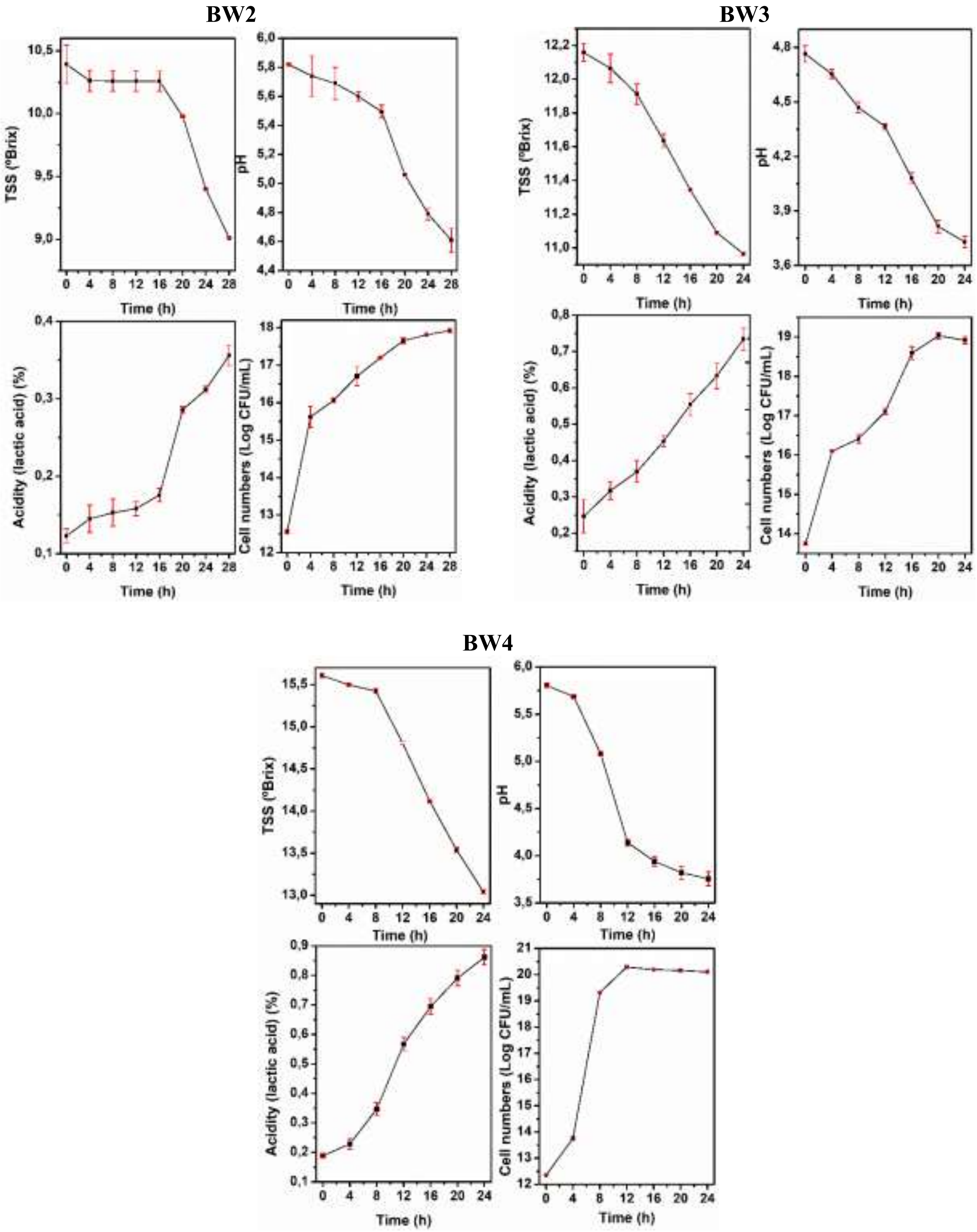

Figure 1. Monitoring of lactic acid fermentation, total soluble solids content (SST), pH, titratable acidity expressed as lactic acid, and cell viability (Log CFU mL^{-1}) to differenciate beer formulations.

The longer adaptation time for LAB in the BW2 formulation was reinforced by the TSS content, which started at 10.40 °Brix, and within the 20-h fermentation interval, they did not show an expressive consumption of the available sugars. Due to this consumption rate, low levels of lactic acid production were observed within the same fermentation period (approximately 0.28%). For this formulation, lactic acid fermentation was followed for 28 h, longer time compared to the other formulations, which lasted 24 h. Therefore, lactic acid fermentation is not favored when having a wort with a °Brix < 10 to craft Berliner Weisse type beers.

In the BW3 formulation, lactic acid fermentation took 24 h, which is considered desirable. From the graphs of fermentation (Figure 1), a linear trend was inferred, which shows regular and increasing adaptation by LAB, followed by a decrease in TSS and pH with an increase in acidity and cell number. This behavior led to a more stable fermentation for this formulation than for BW2 and BW4. This behavior may have contributed to a more acceptable profile of the palate, as demonstrated by the sensory analysis.

The BW4 formulation had the highest initial TSS content (15.60 °Brix (Figure 1)). However, in this formulation, there was a slow adaptation by LAB to the medium; that is, in the first eight hours of the process, all parameters, including cell number, remained practically constant. After 8 h, the cell count increase to 19.31 log CFU mL^{-1}. After this adaptation period, the parameters of TSS content and pH showed a constant decrease and the acidity content and cell number showed a relatively linear increase, indicating the "log" phase of LAB.

Physicochemical parameters of craft beer

The physicochemical characteristics of the final product of each beer formulation are summarized in Table 2.

Table 2
Physicochemical characteristics of Berliner Weisse beer formulations with cashew peduncle pulp

Composition	Formulations			
	C1	BW2	BW3	BW4
Color	41.45±1.27^{a}	20.48±1.25^{c}	41.48±2.45^{a}	30.06±0.40^{b}
pH	2.88±0.23^{c}	4.28±0.15^{a}	3.68±0.08^{b}	3.65±0.03^{b}
Turbidity (EBC)	66.75±2.75^{b}	42.75±3.30^{c}	70.08±3.81^{ab}	75.00±4.24^{a}
Diacetyl (ppm)	0.13±0.03^{ab}	0.10±0.03^{bc}	0.15±0.04^{a}	0.07±0.02^{b}
Initial TSS (ºBrix)	12.27±0.59^{c}	9.92±0.41^{d}	13.56±0.74^{b}	14.90±0.52^{a}
Final TSS (ºBrix)	3.99±0.13^{bc}	4.18±0.11^{bc}	3.60±0.42^{c}	4.68±0.13^{a}
Alcohol (%v/v)	3.95±0.07^{a}	3.44±0.26^{b}	3.86±0.05^{a}	4.01±0.07^{a}
Acidity (% in lactic acid)	1.20±0.53^{a}	0.28±0.02^{b}	0.69±0.04^{ab}	0.78±0.18^{ab}

Triplicate mean values. Means and standard deviations followed by the same letters in the same column that indicate no significant differences according to Tukey's test ($p < 0.05$).

The main characteristics of Berliner Weisse beer are its diacetyl content and acidity. In Brazilian legislation, no reference values for this type of product are delineated; however, according to the Beer Judge Certification Program style guide (Gatza et al., 2017), symbiotic fermentation with yeasts and *Lactobacillus* provides unique characteristics to this style, mainly pungent acidity and aroma and flavor that can resemble "butter," resulting from the diacetyl content. These parameters are mainly influenced by lactic acid fermentation, but are also related to the alcoholic fermentation.

In general, the levels of diacetyl content in C1 and BW3 did not differ statistically, with high diacetyl levels of 0.13 and 0.15 ppm, respectively. This was due to the lactic acid profile and, consequently, alcoholic fermentation, which were more stable and regular in BW3 (Figure 1). In C1, which had the same proportion of malt as BW3, there was no lactic acid fermentation, and all the diacetyl formed came from alcoholic fermentation. Thus, it was possible to infer the same stable and regular fermentation profile by the yeast *S. cerevisiae* for both formulations. As previously discussed, BW2 and BW4 had a slower and non-linear fermentation profile; consequently, these formulations showed the lowest values of diacetyl of 0.10 and 0.07 ppm, respectively.

The alcohol content of the formulations showed statistically significant differences, probably because of the different initial and final TSS levels during alcoholic fermentation. Although the fermentation process was conducted such that the four formulations had the same final alcohol content, this did not occur with BW2, as it had the lowest malt content, and consequently, the lowest TSS content (Table 2). Furthermore, BW2 had the lowest acidity and lactic acid content of 0.28%, whereas BW3 had an intermediate value of 0.69% , and BW4 had a higher value of 0.78%. Commercial lactic acid (85% purity) was added to BW1; hence, its acidity content came mainly from this addition, resulting in the highest value of 1.20%.

To the best of our knowledge, there are no Berliner Weisse beers with cashew peduncle pulp. However, there is a Lager-type of craft beer with cashew peduncle pulp (Pereira et al., 2020). In addition to the different classifications by type, the beers also showed differences in physicochemical characteristics, such as the BW2, BW3, and BW4 formulations generally had lower pH, total acidity, and TSS, than those reported by Pereira et al. (2020). However, the alcohol content was similar between the formulations.

Sensory ordering test

Table 3 summarizes the sensory ordering test results for craft beer formulations. For this Twenty trained panelists evaluated four attributes, the data of which were organized in ascending order with respect to the intensity of fruity aroma, sour taste, fruity flavor, and body.

Table 3
Craft beer preferences according to the ordering test

Composition	Formulations			
	C1	BW2	BW3	BW4
Fruity aroma	60[a]	44[a]	46[a]	50[a]
Fruit flavor	60[a]	35[b]	51[a]	54[a]
Sour taste	68[a]	30[c]	47[b]	55[ab]
Body	55[a]	39[a]	49[a]	57[a]

The same letters in a column indicate no significant differences according to the Friedman test ($p < 0.05$).

Fruit and sour flavor attributes differed significantly between BW2 and C1, BW3, and BW4. BW2 was evaluated as the least intense before these attributes. The difference in sour taste for BW2 was expected because this beer had a lower acidity according to physicochemical characterization. Other attributes, such as fruity aroma and beer body, showed no significant differences between the formulations.

Notably, all formulations contained the same amount of cashew peduncle pulp. There was no interference in aroma perception by the evaluators when the co-adjunct was added to beer for the fruity aroma attribute. However, this same concentration managed to elicit a significant difference in the fruity flavor, characterizing the BW2 formulation as the least intense in context of the pulp flavor of cashew fruit in the craft beer.

The beer body attribute can be described as a feeling of “fullness” of the drink in the mouth that can be associated with many factors, such as the relationship between the malts used. Although the difference in the ratio of malts used between the formulations was 5% (Table 1), these ratios were insufficient for the evaluators to detect significant sensorial changes. In addition, acidity was the most influential and relevant attribute of this beer style, which may have interfered with the sensory perception of the beverage body.

In other studies, sensory analysis generally indicated that beer with fruit or spices brings favorable and unique attributes to craft beer, as in the case of Lager beer with cashew peduncle and orange peel (Pereira et al., 2020); Pilsner beer with ginger (Tozetto et al., 2019); Amber ale beer with goji berries (Ducruet et al., 2017); beer with dandelion, nettle, and sage (Hayward et al., 2019); and soursop (Santos et al., 2021).

Conclusion

The physicochemical and sensory parameters of the Berliner Weisse craft beer formulations with cashew apple pulp allowed us to characterize them as products with unique acidic and fruity characteristics. Different proportions of ingredients generated different behaviors with respect to fermentation parameters. We observed different physicochemical properties, especially in the BW2 formulation, which had lower concentrations of malt, TSS, and hops.

However, all formulations presented intensely fruity and acidic sensory parameters.

Using national raw materials that are often considered co-products of the food industry, such as cashew peduncle pulp, has proven to be a viable and attractive alternative for producing Berliner Weisse-style beer with national characteristics and versatility in physicochemical and sensorial parameters. This feature makes the cashew pseudofruit as a remarkable co-product of the food industry and artisanal beer production.

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