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Application of Pau-tenente (*Quassia amara* L.) as Hop Replacement in Brazilian Low-bitter Beer

Guilherme L. Schuina[a,*], Arturo Solis-Méndez[b], Mariana Molina-Quintero[a], João O. F. Quelhas[a], Mariana D. Oliveira[a], Vanildo L. Del Bianchi[a]

[a] Postgraduate Program in Food Science and Engineering, São Paulo State University, São José do Rio Preto, São Paulo, Brazil
[b] Department of Food Engineering and Technology, São Paulo State University, São José do Rio Preto, São Paulo, Brazil.
glschuina@hotmail.com

Beer is a fermented beverage obtained from barley malt and hop under the action of appropriate microorganisms. Hop is typically used to confer bitterness and flavour to beer. Due to its geographical location, Brazil hosts diverse plant species, many of which are used in food applications. The aim of this study was to evaluate the use of the pau-tenente as a substitute for hops in American lager beer production. Five pau-tenente concentrations were tested (from 0.1 to 0.5 g L^{-1}) against standard hopped beer and a commercial beer. Physicochemical (apparent extract, original extract, alcohol content, pH, colour, turbidity, apparent attenuation, and bitterness) and sensory (acceptance and ideal bitterness intensity) analyses were performed Pau-tenente was not found to affect beer physicochemical characteristics and a low concentration use (up to 0,2 g L-1) yields beers with suitable sensorial parameters. Higher pau-tenente concentrations increase the sensory rejection of beers. Hence, the use 0.1 g L^{-1} pau-tenente can be a viable alternative to produce American lager beers with low bitterness.

1. Introduction

Beer is a fermented beverage obtained from barley malt and hop under action of appropriate microorganisms (Meussdoerffer, 2009). Beer may contain other ingredients and substitutes such as fruits, cereals, spices, and condiments providing the beverage its peculiar sensory characteristics.

Brazil is one of the largest beer producers of the World, together with United States and China (Coelho-Costa, 2018). American lager, popularly known as Pilsen, is the most produced and consumed type of beer in Brazil. This beer is generally formulated with water with low hardness, and an original gravity content between 11 and 13.5 Plato, resulting in an alcohol content of 3 to 5%. Pilsen malt, a small amount of hops and, optionally, adjuncts are used in the production. Major sensory characteristics of this beer type are its refreshing and well-rounded taste, bright and clear colour, and a thick and persistent foam (Strong and England, 2015).

Hop is a typical plant of cold regions, and therefore difficult to cultivate in Brazil. Its ideal cultivation area is between 35 º and 55 º, both in the Southern and Northern Hemispheres (Morais, 2015). Female inflorescence cones of the plant are employed for beer production. These cones contain resins and essential oils that give bitterness and hop flavour to the beer (Oladokun et al., 2016).

Due to its geographical location, Brazil has a rich biodiversity enabling the growth of a diverse array of plant species. Many of these plants are used in food, cosmetics, medicine, and folk medicine applications (Funari & Ferro, 2005). Bitter plants are a part of this biodiversity. These plants are generally consumed in the form of aqueous infusions (teas) in traditional medicine applications.

Quassia amara L. is a shrub that can reach seven meters in height. Popularly known as pau-tenente or quassia amarga -among others-, it is a typical Brazilian plant that can be found in South America (Macedo et al., 2005).

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In folk medicine, quassia is used in diseases of liver, stomach, headache, skin blemishes, among others (Silva, 2015). It is potentially effective in the treatment of diabetes as well (Bieski et al., 2012). This non-toxic plant has an intense bitter taste that may be around 50 times greater than quinine (Teixeira et al., 2014).
The aim of this study was to evaluate the use of pau-tenente (PT) as a total hops substitute in American lager beer production.

2. Material and Methods

2.1 Materials

Pilsen Malt (Agrária®, Brazil), Saaz hops (Barth-Hass Group®, Germany), Saflager S-23 yeast (Fermentis®, France), and pau-tenente (bark) were purchased locally and brought to the Bioprocess Laboratory of the Department of Food Engineering and Technology, Institute of Biosciences, Humanities and Exact Sciences of the São Paulo State University, São José do Rio Preto Campus for analysis.

2.2 Beer production

Ground malt and water were mixed in a mashing tun at a ratio of 1:4. They were submitted to multiple infusion mashing processes (62ºC, 40 minutes; 72ºC, 20 minutes). At the end of mashing, the wort was filtered. The resulting sweet wort was brought to boil, and as soon as it began boiling, hops (standard beer) or pau-tenente (test beer) were added. After 60 minutes of boiling, wort was rapidly cooled to inoculation temperature and subjected to circular stirring to promote trub sedimentation. Sedimented material was removed, and the inoculated wort was fermented.
The fermentation was carried out at 12 ºC for seven days in closed plastic vats (10 L) equipped with a hydraulic stopper. Following fermentation, excess accumulated yeast at the bottom of the vat was removed, and then, the maturation occurred, lasting for 15 days at 0 °C. Mature beers were transferred to glass bottles along with a 6 g L^{-1} sugar solution and kept for seven days at 20 °C to promote carbonation of the product. After this period, the beer was analysed.

2.3 Standard Analysis

Original gravity, final gravity, alcohol content, pH, colour, bitterness, turbidity, and apparent attenuation analyses were performed according to the European Brewery Convention methodology (EBC, 2010).

2.4 Sensory Analysis

The sensorial acceptance of samples for five sensorial attributes (aroma, flavour, colour, and overall acceptance) was made using a structured hedonic scale of nine points (9 = Extremely liked and 1 = Extremely disliked) (Reis & Minim, 2010). The ideal bitterness intensity was measured along with sensorial acceptance using a structured nine-point scale (1 = Extremely less bitter than ideal, 5 = Ideal bitterness, and 9 = Extremely bitter than ideal) (Meilgaard et al., 1999).
The sensory analysis panel consisted of 100 untrained evaluators who received the samples in monadic form, coded with three digits and randomized to avoid bias. The experimental design consisted of complete blocks, i.e., all evaluators analysed the samples using the scales described above. The evaluators were asked to drink a glass of room-temperature water to rinse their mouth before each evaluation.
The project was approved (Process number: 51957715.7.0000.5466) by the Committee of Ethics in Research Involving Humans of Institute of Biosciences, Humanities and Exact Sciences of the São Paulo State University (IBILCE/UNESP)

2.5 Data Analysis

The beers were produced in three replicates and the analyses were performed in triplicates. Physicochemical analyses of beer samples and their sensorial attributes were done by performing Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test when $P < 0.05$. The results were further compared using an internal preference map, which was built using cluster analysis followed by multidimensional scaling. All analyses were performed at 5% significance level using Statistica 10.0 software (StatSoft Inc., USA).

3. Results and discussion

3.1 Physicochemical characterization

All beers produced in this study presented similar original gravity, between 12.3 and 12.5 ºP, with no significant differences among them (Table 1). Each sample was observed to be in the intended beer style.

According to The Beer Judge Certification Program (BJCP), American lager beers have an original extract between 10 and 12.5 ºP approximately (Strong and England, 2015).

Table 1: Physicochemical characteristics of the beers

Samples	Original Gravity (ºP)	Final Gravity (ºP)	Apparent Attenuation (%)	Alcohol Content (%)	Colour (EBC)	Turbidity (EBC)	Bitterness (IBU)
Standard	12.4 (0.15)[a]	3.3 (0.09)[a]	73.3 (1.1)[a]	4.9 (0.14)[a]	9.50 (0.01)[ab]	6.20 (0.383)[a]	8.63 (0.35)
PT 0.1 g L^{-1}	12.5 (0.10)[a]	3.4 (0.18)[a]	72.8 (1.6)[a]	4.9 (0.16)[a]	10.3 (1.04)[ab]	10.2 (0.19)[b]	-----
PT 0.2 g L^{-1}	12.4 (0.21)[a]	3.3 (0.14)[a]	73.1 (1.4)[a]	4.9 (0.15)[a]	11.0 (0.04)[a]	10.1 (0.06)[b]	-----
PT 0.3 g L^{-1}	12.3 (0.15)[a]	3.3 (0.18)[a]	73.2 (1.6)[a]	4.9 (0.18)[a]	9.10 (0.03)[b]	8.00 (0.01)[c]	-----
PT 0.4 g L^{-1}	12.3 (0.26)[a]	3.4 (0.18)[a]	72.6 (1.6)[a]	4.9 (0.23)[a]	10.2 (0.95)[ab]	13.1 (0.07)[d]	-----
PT 0.5 g L^{-1}	12.4 (0.15)[a]	3.5 (0.32)[a]	71.8 (2.7)[a]	4.9 (0.13)[a]	10.9 (0.09)[a]	16.1 (0.06)[e]	-----
Commercial	-----	-----	-----	4.6 (0.05)[a]	6.40 (0.12)[c]	1.36 (0.05)[f]	7.10 (0.44)

* Mean (standard deviation); ** There was a statistically significant difference between the means followed by the different letters in columns as reported by Tukey's multiple comparison test at $P < 0.05$.

There was also no significant variation between the evaluated samples with respect to the apparent extract of beers studied, with values between 3.3 and 3.5 ºP. The same was observed for apparent attenuation as well (no significant difference): the values ranged between 71.8 and 73.3% among the samples. In a brewing process, attenuation of around 70% is common (Mayer et al., 2016; Rogers et al., 2016). This shows that using pau-tenente instead of hops did not cause any inhibition to the yeast used, and thus did not affect the fermentation step.

Likewise, alcohol content - another variable associated with the fermentation process - also did not differ significantly among analysed samples. Strong and England (2015) recommend that American lager beers should have an alcohol content of 4.2 and 5.3 %. All of the samples fit within the style proposed in their study.

The colour and turbidity presented a significant difference between the samples. Commercial sample presented the brightest colour and lowest turbidity, with values of 6.4 and 1.36 EBC, respectively. These low turbidity values can be attributed to the superior filtration methods to which commercial beers are submitted (Cimini; Moresi, 2018; Marques et al., 2017).

The samples produced in this study showed higher colour and turbidity than the commercial beer. The higher turbidity is directly attributed to the lack of filtering. The beer with 5 g L^{-1} of pau-tenente showed the highest values for both parameters, with a colour and turbidity of 10.9 and 16.1 EBC, respectively. Hop-containing beer presented the closest turbidity to the commercial, although it did not differ significantly from some samples with pau-tenente, such as those containing 0.1 and 0.3 g L^{-1} of the plant.

During boiling, the complexation of polyphenols in hops with the proteins in wort occurs. This process results in the formation of trub, which decants and is withdrawn from the process (Jakubowski et al., 2016). This fact may explain the lower turbidity of the sample containing hops compared with samples containing pau-tenente.

The beer bitterness measure expresses the number of alpha-acids of hops present in beer. For this reason, it was only quantified for standard and commercial beer. Values were between 7 and 9 IBU, in line with the bitterness characteristic of the beer style.

The concentrations of pau-tenente evaluated in this experiment were determined via sensory pre-tests by observing which concentrations showed similar sensorial bitterness to those obtained from standard and commercial beer.

Physicochemical parameters are important to describe beer quality (Liguori et al., 2015). The use of pau-tenente as hops substitute clearly did not cause any damage to physicochemical characteristics since higher turbidity is easily corrected in the industrial process with the use of a filtration step.

Similar results were found in other studies using other plants instead of hops. Okafor et al. (2016) studied tropical plants of Nigeria to replace hops, and also did not observe significant variation in the physicochemical parameters. Adenuga, Olaleye, and Adepoju (2010) also did not observe alterations in physicochemical characteristics in beers of sorghum elaborated with different extracts of African plants.

3.2 Sensory acceptance

Sensorial acceptance results show that standard and commercial beers were in general the most accepted (Table 2). Sensorial acceptance of the sample containing 0.1 g L^{-1} pau-tenente approached those of standard and commercial beers. There was an inverse correlation between pau-tenente concentration and sensorial acceptance.

The commercial beer had the highest overall acceptance and aroma values, with scores of 7.17 and 6.66, respectively. However, the values did not differ significantly from standard and the 0.1 g L^{-1} pau-tenente sample.
0.1 g L^{-1} pau-tenente sample showed lower flavour acceptance than standard and commercial samples, as 0.2 to 0.4 g L^{-1} pau-tenente beers.

Table 2: Sensory acceptance scores of the beers

Samples	Colour	Aroma	Flavour	Overall acceptance	Ideal Bitterness
Standard	6.88 (1.57)^{a}	6.77 (1.54)^{ab}	6.79 (1.82)^{a}	6.80 (1.49)^{a}	4.52 (1.33)^{b}
PT 0.1 g L^{-1}	5.83 (1.77)^{b}	6.22 (1.62)^{ab}	5.56 (1.68)^{b}	5.56 (1.74)^{a}	5.05 (1.79)^{ab}
PT 0.2 g L^{-1}	5.82 (1.74)^{b}	5.60 (1.72)^{bc}	5.35 (2.19)^{bc}	5.54 (1.97)^{ab}	5.17 (1.76)^{ab}
PT 0.3 g L^{-1}	5.81 (1.76)^{b}	5.96 (1.77)^{bc}	5.12 (209)^{bc}	5.40 (1.81)^{b}	5.16 (1.76)^{ab}
PT 0.4 g L^{-1}	6.09 (1.40)^{b}	5.37 (1.95)^{c}	4.74 (2.00)^{bc}	5.09 (1.81)^{b}	5.57 (2.01)^{a}
PT 0.5 g L^{-1}	5.74 (1.92)^{b}	5.41 (1.74)^{c}	4.62 (1.78)^{c}	4.85 (2.09)^{c}	5.62 (2.22)^{a}
Commercial	7.35 (1.49)^{a}	6.66 (1.69)^{a}	6.79 (1.59)^{a}	7.17 (1.46)^{a}	4.50 (1.07)^{b}

* Mean (standard deviation); ** There was a statistically significant difference between the means followed by the different letters in columns as reported by Tukey's multiple comparison test at $P < 0.05$.

There was a significant difference between the colour acceptance of pau-tenente and commercial/standard beers. Table 1 shows that colours of all samples produced were similar to each other. Considering the fact that sensory acceptance test was carried out with consumers (untrained tasters), colour acceptance results were possibly affected by acceptance of flavour and aroma.
The ideal bitterness results show that according to consumers, all samples were close to the ideal bitterness. This indicates that the lowest sensory acceptance obtained by beers containing 0.3 to 0.5 g.L^{-1} of pau-tenente is correlated to the flavour, and not to the bitterness of the samples.
Low pau-tenente concentrations up to 0.2 g L^{-1} allowed the production of beer with statistically equivalent results to standard and commercial beers with good global acceptance. Thus, pau-tenente can be an alternative to hops in beer production.
Clustering analysis for global samples acceptance (Figure 1) shows the Euclidean distance between samples. Accordingly, standard and commercial samples were clustered within the same group, separated from pau-tenente samples.

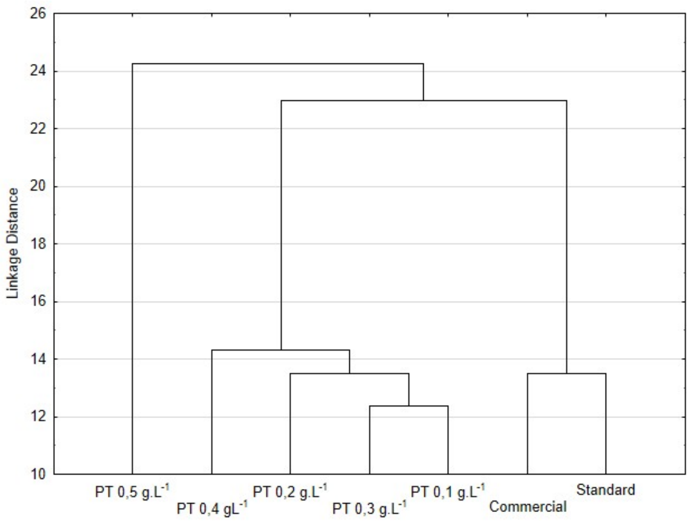

Figure 1: Dendrogram for the global acceptance of beers

The internal preference map (Figure 2) shows the attitude of consumers with respect to samples evaluated in this study. Scatter points represent consumers, whereas demarcated points denote the samples. The closer samples are to the consumers, the higher their sensory acceptance.

As in the dendrogram, standard and commercial samples are close to each other. Pau-tenente beers were placed apart from commercial and standard beers, showing that they were perceived differently by consumers.

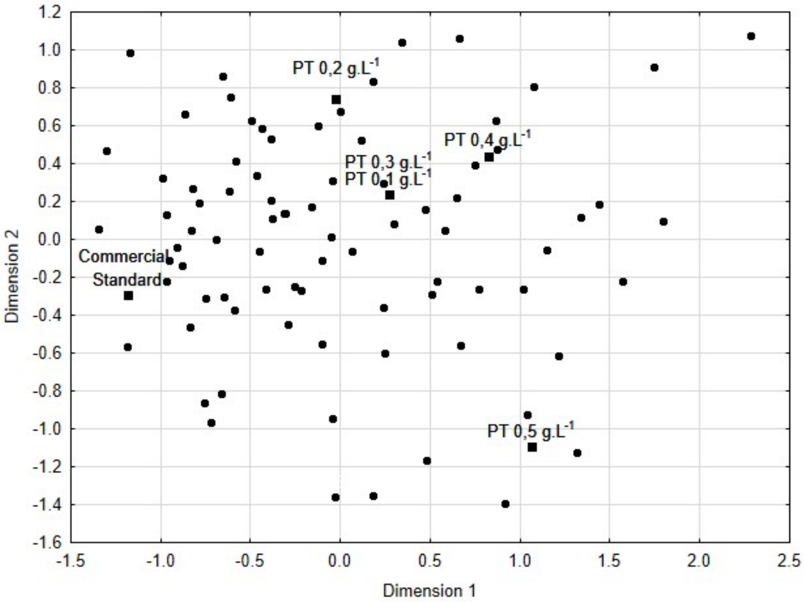

Figure 1: Internal preference map for the global acceptance of beers

However, many consumers are placed near sample containing 0.1 g L^{-1} pau-tenente. This shows that, despite being rated differently from hops beers, pau-tenente beers show good sensory acceptance and potential for application in an industrial process.

The sample with 0.5 g L^{-1} pau-tenente is the most distant from consumers. The increase in the concentration of pau-tenente leads to a reduction in sensorial acceptance of the beer.

4. Conclusions

The use of pau-tenente did not affect the fermentation process or the physicochemical characteristics of the beers. The use of low amounts of pau-tenente, of up to 0.2 g L^{-1}, allows beer production with sensory acceptance similar to beers produced with hops. The increase in pau-tenente concentration results in a lower sensorial acceptance. Production of beer with low bitterness with pau-tenente, instead of hops, is possible.

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