CAPÍTULO 10

GERVÃO-ROXO (*STACHYTARPHETA CAYENNENSIS*) AS A BITTER INGREDIENT IN BEER

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ABSTRACT: Brazil is one of the world's largest beer producers, and its beer market grows every year. Hops (*Humulus lupulus*), a raw material used in beer production and responsible for the bitter taste, has a significant economic impact on beer production due to its importation. The objective of this work was to use gervão-roxo (*Stachytarpheta cayennensis*) as a bitter ingredient in beer production, replacing hops. Chemical composition analysis, bioactive compounds, antioxidant activity, and bitterness of gervão-roxo and hops were carried out. Beers with 0, 25, 50, and 75% hop replacement were prepared. Beers made with gervão-roxo were evaluated by consumers in sensory analysis tests. Differences in the chemical composition, bioactive compounds, and bitterness between gervão-roxo and hops were observed. However, the physicochemical characteristics and bioactive compounds of the final product showed similarities, differing only in bitterness due to the intrinsic factors of the raw material. The results obtained, along with sensory acceptance data, indicate that gervão-roxo can be used as a bitter ingredient in beer production.

KEYWORDS: alcoholic fermentation; bitter ingredient; hops; sensory analysis.

GERVÃO-ROXO (*STACHYTARPHETA CAYENNENSIS*) COMO INGREDIENTE AMARGO EM CERVEJA

RESUMO: O Brasil é um dos maiores produtores de cerveja do mundo e seu mercado de cervejeiro cresce a cada ano. O lúpulo (*Humulus lupulus*), matéria-prima utilizada na produção de cerveja e responsável pelo sabor amargo, tem impacto econômico significativo na produção de cerveja devido à sua importação. O objetivo deste trabalho foi utilizar o gervão-roxo (*Stachytarpheta cayennensis*) como ingrediente amargo na produção de cerveja, em substituição ao lúpulo. Foram realizadas análises de composição química, compostos bioativos, atividade antioxidante e amargor do gervão-roxo e do lúpulo. Foram elaboradas cervejas com 0, 25, 50 e 75% de substituição de lúpulo. As cervejas elaboradas com gervão-roxo foram avaliadas por consumidores em testes de análise sensorial. Foram observadas diferenças na composição química, compostos bioativos e amargor entre gervão-roxo e lúpulo. As características físico-químicas e de compostos bioativos do produto final apresentaram semelhanças, diferindo apenas no amargor devido aos fatores intrínsecos da matéria-prima. Os resultados obtidos, juntamente com os dados de aceitação sensorial, indicam que o gervão-roxo pode ser utilizado como ingrediente amargo na produção de cerveja.
PALAVRAS-CHAVE: fermentação alcoólica; ingrediente amargo; lúpulo; análise sensorial.

INTRODUCTION

Beer is defined as a drink obtained from the alcoholic fermentation of brewer's wort, derived from barley malt and drinking water, by the action of yeast, with the addition of hops (EßLINGER, 2009). Hops (*Humulus lupulus*) is the ingredient that gives the drink its characteristic aroma and bitterness. Only its unfertilized female flowers are used in the production of beer; they are rich in yellow glands containing lupulin, a resinous material with a bitter taste, where resins, essential oils, anthocyanins, tannins, and α-acids (humulones) predominate. Originally, hops were used to preserve beer, but over time, it gained importance in two sensory qualities: the aromatic, which comes from essential oils, and the bitter taste, which comes from lupulin, mainly from humulones. In the wort cooking stage, hop α-acids are isomerized into iso-α-acids, with even more bitter and soluble characteristics (DURELLO; SILVA; BOGUSZ JR., 2019). This bitterness depends on the quantity of hops added to the beer, which is measured in International Bitterness Units - IBU (EßLINGER, 2009).

A major bottleneck in the Brazilian brewing industry is the production of hops. A large part of the hops used in beer production is imported, which increases the cost of production. The growth of the brewing industry in Brazil creates a new research niche that seeks new ingredients for beer formulation, ingredients that can replace hops. In this search for the appropriate matrix, it is important that the chosen plant acts in the processing of beer in a similar way to hops, in the precipitation of proteins, conferring characteristics of bitterness, antioxidant action, and the influencing on the formation and stability of the foam.

Recent studies show that bitter ingredients used as substitutes for hops, in addition to imparting bitterness to the beverage, also enrich it with antioxidant compounds and obtain sensory acceptance from consumers. Rubim (*Leonurus sibiricus* L.) and Mastruz (*Chenopodium ambrosioides* L.) (LAZZARI et al., 2022, 2023), carqueja (*Baccharis trimera* (Less.) DC. Asteraceae) (SCHUINA et al., 2020), sage (*Salvia officinalis*), and dandelion (*Taraxacum*) and nettle (*Urtica dioica*) (HAYWARD; WEDEL; MCSWEENEY, 2019) have been used as bitter ingredients in beer.

The phytochemical characteristics of hops can be found in other ingredients that can be used as substitutes for hops. The gervão-roxo (*Stachytarpheta cayennensis* (L.C. Rich) Vahl) is a fibrous subshrub used in folk medicine for the treatment of stomach ulcers, pain, fever, and diarrheal. It has anti-inflammatory, analgesic, gastroprotective, and antimicrobial activity. This plant is characterized by the presence of alkaloids, glycosides (verbenalin and verbenin), tannins, saponins, flavonoids, steroids, quinones, phenolic compounds, and glycyrrhizin acid as chemical constituents (SHAH, 2021).

Based on the phytochemical composition of gervão-roxo and the need for Brazilian breweries to seek new alternatives to replace hops, this work aims to evaluate the physical-chemical properties and bioactive compounds of gervão-roxo (*S. cayennensis*) and use them as a bitter ingredient in beer production.

MATERIALS AND METHODS

Materials and reagents

Gervão-roxo (*S. cayennensis*) was obtained in the northwest region of Rio Grande do Sul, Brazil (27° 56’ 38” S, 52° 55’ 23” W). Pilsen malt (Agrária, Brazil) and Hallertauer Tradition pellet hops (Barth-Hass Group, Germany) were generously donated by Industrial Norte Paranaense de Bebidas (INBEB; Londrina, PR, Brazil). Safale US-05 yeast was sourced from the Fermentis Division of S. I. Lesaffre (Marcq-en-Baroeul, France).

Folin-Ciocalteu reagent, gallic acid, 2,2’-azino-bis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), quercetin, methyl alcohol, hydrochloric acid, trichloroacetic acid, phosphate buffer, sodium carbonate, potassium persulphate, and β-carotene (purity ≥ 95%, synthetic, type II and crystalline) were obtained from Sigma Aldrich (St. Louis, Missouri, USA). Aluminium chloride, potassium ferricyanide, and ferric chloride were of analytical grade.

Characterization of bitter ingredients

Initially, the gervão-roxo was washed and sanitized in a sodium hypochlorite solution (200 ppm/15 min) and subsequently dehydrated in an oven with air circulation at 55 °C until a constant weight was achieved. The gervão-roxo was then crushed, and its granulometry was standardized (60 mesh). Both gervão-roxo and hops samples had their chemical composition evaluated for moisture, mineral content, fibre, lipids, carbohydrates, and proteins (AOAC, 1990)

Methanolic extracts (1:10) of gervão-roxo and hops were used for bioactive compounds and antioxidant activity assays. An aliquot of 300 μL of the methanolic extract was added to 150 μL of aluminium chloride (50 g/L) and 2250 μL of methanol to determine the total flavonoids. The absorbance was measured at 425 nm, and the results were expressed in quercetin equivalents (BURIOL et al., 2009).

Total polyphenol content was determined according to Singleton & Rossi (1965). An aliquot of 125 μL of methanolic extract was mixed with 125 μL of Folin-Ciocalteu (50%) and 2250 μL of sodium bicarbonate (28g/L). The absorbance was measured at 725 nm, and the results were expressed as gallic acid equivalents.

Antioxidant assays for the capture of the ABTS radical (2,2 AZINO BIS [3-ethylbenzothiazoline, 6-sulfonic acid]) and DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and antioxidant capacity by the iron ion reduction power (FRAP) were performed. The percentage of ABTS radical capture was determined by reacting 40 μL of methanolic extract with 1960 μL of ABTS* solution (7 mM) and then measuring the absorbance at 734 nm using a spectrophotometer) (RUFINO et al., 2010).

The DPPH assay was determined by reacting 150 μL of methanolic extract with 2850 μL of DPPH solution (0.06 mM). After 30 minutes of incubation in the dark, the absorbance reading was performed at 515 nm, and the percentage of antioxidant activity was calculated (Brand-Williams et al., 1995).

FRAP was determined by the reaction of 250 μL of methanolic extract with 1250 μL phosphate buffer (0.5 mol/L and pH 7) and 1250 μL potassium ferrocyanide (1%). The solution was then kept at 50 °C for 20 minutes. After cooling, 1250 μL trichloroacetic acid (10%) was added, the samples were centrifuged at 3000 rpm for 10 minutes, the supernatant was recovered, and 0.5 mL of iron chloride (0.1%) was added. The absorbance was read at 700 nm, and the results were expressed as gallic acid equivalents (ZHU et al., 2002).

Global bitterness was determined according to Philpott *et al.* (1997) with modifications. The extraction of bitter substances from gervão-roxo and hops was performed with 4 mL of 2,2,4-trimethylpentane in samples acidified with 200 μL of 3 mol/L HCl, followed by stirring for 15 minutes and centrifugation (MPW 351R, Warszawa, Poland) at 3000 g for 10 minutes. The bitterness of the supernatant was measured at 275 nm, using a spectrophotometer (EvolutionTM 300; Thermo Fisher Scientific, Cambridge, UK), and expressed in IBU, calculated by Eq. 1, where $A_{(275\ nm)}$ is the absorbance at 275 nm:

$$\text{Bitterness (IBU)} = A_{(275\ nm)} \times 50 \qquad (1)$$

Beer preparation

The brewing wort was prepared from a mixture (1:4) of crushed malt and water. It was initially heated to 44 °C for 30 minutes, followed by a temperature increase to 52 °C for 20 minutes, another temperature increases to 70 °C for 30 minutes, and finally maintained at 76°C for 5 minutes. The entire mashing process was conducted with agitation, at the end the wort was filtered. Subsequently, the wort was concentrated at 98 °C for 60 minutes to reach 12.5 °Brix. Then, hops and gervão-roxo were added, as shown in Table 1.

Beers	Hop replacement (%)	Hops (g)	Gervão-roxo (g)
B00	0	1.32	0.00
B25	25	0.99	1.47
B50	50	0.66	2.94
B75	75	0.33	4.40

Table 1. Percentage of substitution of hops for gervão-roxo.

Four top-fermentation beer formulations were prepared with a partial replacement of hops. The amount of gervão-roxo added was calculated based on the overall bitterness of hops and gervão-roxo, determined as previously described.

After boiling, the wort was cooled to 25 °C, filtered, and yeast (Fermentis - SafAle™ US-05) was added according to the manufacturer's recommendation (0.5 g/L). Fermentation took place at 18 °C for 7 days. After the fermentation period, the fermented product was matured at 2 °C for 7 days. After this period, the fermented product was packaged in 600 mL amber bottles, cleaned, sanitized, and labelled. The carbonation of the fermented product was achieved by adding sucrose (3 g/L) at a temperature of 23 °C for 7 days. At the end of carbonation, the fermented product was pasteurized at 70 °C for 15 minutes to obtain pasteurized beer.

Beer characterization

Decarbonated beer samples (centrifuged at 3000 rpm for 10 minutes) were evaluated for initial soluble solids content, pH, total acidity, fixed acidity, and volatile acidity (INSTITUTO ADOLFO LUTZ, 2008). To determine the colour, 5 mL of each decarbonated sample was transferred to a cuvette, and the spectrophotometer reading was performed at 430 nm, using distilled water as a blank. The colour was expressed in EBC (European Brewery Convention) according to Eq. 2, where A is the absorbance, and 25 is the conversion factor (EUROPEAN BREWERY CONVENTION, 2007):

$$\text{Colour (EBC)} = A \times 25 \quad (2)$$

The alcoholic strength (AC) of the beers was determined from the initial density (ID) and final density (DF) of the beer wort, following Eq. 3 (PAPAZIAN, 2014):

$$AC (\%) = 131.25 \times (ID - FD) \tag{3}$$

An aliquot of each decarbonated sample was used for the analysis of bioactive compounds, antioxidant activities, and overall bitterness (without the need for the extraction of bitter compounds) as previously described.

Consumer acceptance

A consumer trial (n = 67) was conducted at the State University of Maringá. Participants were recruited through online advertisements. The analysis was approved by the Research Ethics Committee at State University of Maringá (CAAE: 39847220.8.0000.0104). Before the analysis, participants were asked to complete a questionnaire consisting of questions about their demographics, interest in beer, knowledge about beer, and beer consumption habits.

Beers were served in acrylic glasses coded with random three-digit numbers. Thirty millilitres of the beer samples were served at 4 ºC. Participants evaluated all four beer samples. Water was provided for mouth rinsing between beer sample tastings. The samples were presented in random order. Consumers were instructed to take a mouthful of each beer and were free to finish the full sample of each beer. They were asked questions regarding their appraisal of colour, aroma, flavour, and overall acceptance on a structured 9-point hedonic scale l(GARCÍA-GÓMEZ et al., 2019; MEILGAARD, 1999). A medium score was excluded, following Furnols *et al.* (2008). A structured 5-point scale was used to evaluate bitterness intensity (1 = extremely intense to 5 = little intense) (MINIM, 2010). Purchase intention of beers was assessed at the same time using a three-point scale (VITAL et al., 2018), and results were expressed as a percentage.

Statistical analysis

The experiment was performed three times, and the analyses were conducted in triplicate. The data were subjected to analysis of variance (ANOVA) and Tukey's test at the 5% level. Multiple Correspondence Analysis (MCA) was performed using Statistica 10.0 software (Start Soft, Inc., USA), and Principal Component Analysis (PCA) was conducted with Origin® 2018.

RESULTS AND DISCUSSION

Chemical characteristics of bitter ingredients

Gervão-roxo is an erect, perennial, branched, fibrous subshrub, highly resistant to traction (SHAH, 2021), while the hops used in the preparation of beers are produced from unfertilized female flowers of *H. lupulus* (BIR; NORWOOD, 2020). These intrinsic characteristics of each of these plants undoubtedly influence their chemical composition. As observed in Table 2, all chemical composition parameters, except for carbohydrates and fibre, are found in higher concentrations in hops. Nutrients from the bittering ingredients may contribute to the fermentation process, as they increase the nutritional value of the wort, promoting yeast reproduction. Yeasts require minerals, nitrogen sources (proteins), and energy sources (carbohydrates and lipids) to facilitate a more efficient production of carbon dioxide and ethanol (AQUARONE et al., 2001). It is important to note that a wort rich in fibre aids in the beer clarification process, as fibres have a filtering capacity (ARTIT KONGKAEW, 2012; CELA et al., 2020).

Assays	Gervão-roxo	Hops
Chemical composition (%)		
Fiber*	20.94 ± 1.17^{a}	17.20 ± 0.14^{b}
Lipids	3.64 ± 0.19^{b}	15.51 ± 0.66^{a}
Ash	6.33 ± 0.05^{b}	8.14 ± 0.14^{a}
Proteins	13.09 ± 0.29^{b}	14.57 ± 0.26^{a}
Moisture	8.73 ± 0.08^{b}	10.21 ± 0.01^{a}
Carbohydrates	68.37 ± 0.38^{a}	34.32 ± 0.88^{b}
Bioactive compounds		
Flavonoids (mgQE/g)	3.30± 0.17^{b}	14.66 ± 2.69^{a}
Total polyphenols (mgGAE/g)	0.69 ± 0.03^{b}	70.74 ± 1.90^{a}
ABTS (%)	10.75 ± 2.20^{b}	17.85 ± 0.82^{a}
DPPH (%)	17.83 ± 2.04^{b}	63.96 ± 0.39^{a}
FRAP (mgGAE/g)	0.96 ± 0.15^{b}	43.47 ± 3.61^{a}
Bitterness (IBU)	9.00 ± 0.02^{b}	40.00 ± 0.10^{a}

Table 2. Chemical composition and content of bioactive compounds of gervão-roxo and hops. Results are expressed as mean ± standard deviation. Different letters on the same line indicate significant differences. *The fiber content is included in the total carbohydrate amount. IBU: International Bitterness Units; QE: quercetin equivalent; GAE: gallic acid equivalent; ABTS: antioxidant analysis using the ABTS method; DPPH: antioxidant analysis using the DPPH method; FRAP: antioxidant analysis using the reducing power of the iron ion.

The intrinsic factors of gervão-roxo and hops also influenced the concentrations of bioactive compounds and antioxidant capacities. The flowers, which are the parts of the plant used to manufacture hops, contain significant amounts of lupulin, a substance that contains essential oils, resins, polyphenols, tannins, and α-acids. These two substances are responsible for the bitterness of hops and are bioactive compounds with antioxidant activity (OLADOKUN et al., 2016). Therefore, they justify the differences in bioactive compounds and existing antioxidant capacities between hops and gervão-roxo, as shown in Table 2, since the bittering ingredient obtained from gervão-roxo consisted of flowers, stems, and leaves.

The presence of bitter substances such as α-acids, tannins, and lupulin in hops may explain the difference in bitterness between hops and gervão-roxo, as observed in Table 2. Since gervão-roxo had less bitterness, the bitterness index was used as the parameter for beer production when substituting hops with gervão-roxo. To achieve a beer with similar characteristics to a beer made with hops, enough gervão-roxo was used to obtain the same level of bitterness, as shown in Table 1.

Physicochemical properties and bioactive compounds of beers

The organic and inorganic acids found in beers are of great importance for the sensory properties of the drink. They originate from the raw materials used, are derived from compounds produced during the fermentation process, and result from the release of substances due to yeast autolysis (CIOCH-SKONECZNY et al., 2023). In Table 3, it is possible to observe that the fixed acidity remained unchanged regardless of the level of addition of gervão-roxo. This type of acidity comes from the acids contained in the raw materials and other ingredients (CIOCH-SKONECZNY et al., 2023). The unaltered behaviour of fixed acidity indicates that the replacement of hops with gervão-roxo does not interfere with the acid composition of the wort, thus not hindering the fermentation process.

When observing the total acidity of the beer samples, it was verified that it is influenced by volatile acidity. Samples with higher concentrations of gervão-roxo (B50 and B75) have lower volatile acidity. Volatile acidity in beers originates from the production of acetic acid during fermentation. Low concentrations of acetic acid and, therefore, low volatile acidity is important so that the beer does not have a bitter taste and unpleasant smell (*29*). The other physicochemical parameters, soluble solids, alcohol content, pH, and colour, were not affected by the substitution of hops with gervão-roxo.

Assays	B00	B25	B50	B75
Physicochemical properties				
Total acidity (meq/L)	37.09 ± 0.97[a]	35.08 ± 2.11[ab]	32.34 ± 0.99[c]	33.27 ± 1.10[bc]
Volatile acidity (meq/L)	16.83 ± 2.66[a]	15.49 ± 1.53[ab]	12.05 ± 2.21[c]	13.20 1.55[bc]
Fixed acidity (meq/L)	20.25 ± 2.12[a]	19.59 ± 0.91[a]	20.29 ± 1.28[a]	20.07 ± 0.59[a]
Dry extract (g/L)	49.43 ± 2.38[ab]	48.81 ± 2.76[ab]	52.27 ± 2.50[a]	47.36 ± 2.44[b]
Soluble solids (ºBrix)	6.65 ± 0.16[a]	6.50 ± 0.33[a]	6.65 ± 0.38[a]	6.40 ± 0.66[a]
Alcohol content (%)	5.93 ± 0.55[a]	5.74 ± 0.73[a]	5.58 ± 0.11[a]	5.61 ± 0.69[a]
pH	5.15 ± 0.01[a]	5.18 ± 0.10[a]	5.15 ± 0.12[a]	5.10 ± 0.05[a]
Colour (EBC)	3.60 ± 0.77[a]	3.06 ± 0.29[a]	3.05 ± 0.46[a]	3.36 ± 0.64[a]
Bioactive compounds				
ABTS (%)	55.85 ± 11.01[a]	52.74 ± 2.50[a]	53.55 ± 2.83[a]	61.61 ± 9.07[a]
DPPH (%)	72.54 ± 1.91[b]	72.17 ± 6.53[b]	75.34 ± 3.92[ab]	79.12 ± 2.48[a]
FRAP (mgGAE/L)	65.47 ± 13.01[a]	59.76 ± 3.37[a]	57.87 ± 2.21[a]	60.63 ± 5.37[a]
Total polyphenols (mgGAE/L)	192.33 ± 8.11[a]	192.71 ± 4.17[a]	191.77 ± 5.79[a]	195.31 ± 4.73[a]
Flavonoids (mgQE/L)	15.74 ± 0.83[a]	17.12 ± 2.95[a]	16.00 ± 1.78[a]	15.31 ± 0.79[a]
Bitterness (IBU)	20.53 ± 1.14[a]	14.80 ± 1.20[b]	11.08 ± 1.71[c]	6.45 ± 0.69[d]

Table 3. Physicochemical properties and bioactive compounds of beers. Results are expressed as mean ± standard deviation. Different letters in the same line indicate significant differences. QE: quercetin equivalent; GAE: gallic acid equivalent; ABTS: antioxidant analysis using the ABTS method; DPPH: antioxidant analysis using the DPPH method; FRAP: antioxidant analysis using the reducing power of the iron ion; B00: beer without hop replacement; B25: beer with 25% hop replacement; B50: beer with 50% hop replacement; B75: beer with 75% hop replacement.

The substitution of hops for gervão-roxo did not influence the number of bioactive compounds and antioxidant activity between the samples. The total polyphenols present in beer have an influence on its preservation and shelf life since their oxidation can alter its sensory characteristics, such as colour, aroma, and flavour (ARON; SHELLHAMMER, 2010). The antioxidant activity present in beer is a result of its composition of esters, aldehydes, organic acids, phenols, and other compounds, which are directly related to the quality of the ingredients used in the manufacturing process (VIANA et al., 2021).

It was observed that the samples differed in terms of their bitterness, and this analysis is based on the number of tannins present in the samples. Tannins are polyphenols from plants that contribute to flavour and palatability, being responsible for the astringency and bitterness of beer. The low bitterness value of beers with hops substituted can be justified due to the formation of insoluble complexes of the tannins present in the gervão-roxo with the malt proteins, which precipitated during the boiling of the wort and formed the trub (beer residue) (LAZZARI et al., 2022; SARAIVA et al., 2019).

Consumer trial

The profile of consumers who participated in the sensory analysis is presented in Table 4. Most of the consumers are men (64.18%), and the majority declared themselves to be regular beer drinkers (83.60%). The consumers' ages ranged from 21 to 29 years, with a high level of education and family income. Consumers answered a questionnaire about their preference for beer style and bitterness intensity. Most of the consumers prefer traditional beer (61.20%), which includes beer with malt, hops, and yeast only, while 38.80% prefer craft beer. Both groups enjoy beer with low bitterness (86.56%), as shown in Fig. 1a.

Gender	(%)
Men (n = 43)	64.18
Women (n = 24)	35.82
Frequency of drinking	
Frequent	83.60
Not Frequent	16.40
Age	
21 - 29	100
Education level	
High	100
Family income	
Up to 2 wages	14.93
2 – 6 wages	40.31
6 – 10 wages	22.38
> 10 wages	22.38

Table 4. Sociodemographic characteristics of identifying consumers' profile. Frequent: drink beer once and twice a week; Not frequent: drink beer once and twice a month.

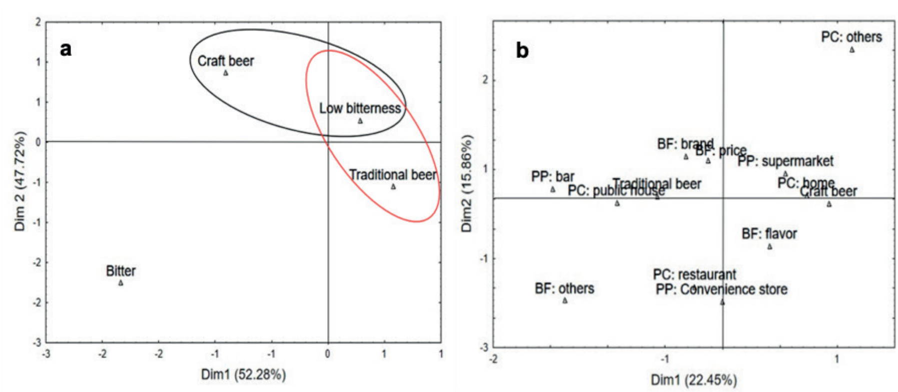

Figure 1. a) Correspondence analysis of preferences towards beers among the testers (n = 67). b) Multiple Correspondence Analysis (MCA) of purchase, consumption preference, and purchasing factors by beer style. PP: place of purchase; PC: place of consumption; BF: buying factor.

A Multiple Correspondence Analysis (MCA) was performed to better understand the relationships between purchase place, consumption place, and buying factors by beer style (Fig. 1b). Traditional beer drinkers prioritize the beer brand and price, while craft beer drinkers emphasize flavour, the search for quality, and product craftsmanship. This gradual shift in consumer habits and purchasing behaviour is leading them to consume beer at home rather than in places traditionally associated with consumption, such as public houses, convenience stores, restaurants, and bars. This trend demonstrates that craft beer can be evaluated as a high-quality beverage and highlights a social subculture that prefers to drink less but better (AQUILANI et al., 2015; KOCH; SAUERBRONN, 2019).

Attributes	**B00**	**B25**	**B50**	**B75**
Colour	6.39 ± 1.56[a]	6.52 ± 1.57[a]	6.02 ± 1.80[a]	6.22 ± 1.65[a]
Aroma	6.77 ± 1.57[a]	6.75 ± 1.60[a]	6.58 ± 1.60[ab]	6.00 ± 1.75[b]
Flavour	6.31 ± 2.10[a]	5.92 ± 2.11[ab]	5.73 ± 2.28[ab]	5.02 ± 2.26[b]
Overall acceptance	6.50 ± 1.74[a]	6.22 ± 1.90[ab]	5.92 ± 1.96[ab]	5.47 ± 2.00[b]
Bitterness intensity	3.94 ± 1.11[a]	3.55 ± 1.05[ab]	3.17 ± 1.21[bc]	2.78 ± 1.07[c]
Purchase intention (%)				
Would certainly buy	28.35	38.80	29.85	26.86
Maybe buy	47.76	35.82	37.31	17.91
Wouldn’t buy	23.88	25.37	32.83	55.22

Table 5. Sensory characteristics and purchase intention of beers. Results are expressed as mean ± standard deviation. Different letters in the same line are significantly different ($p \leq 0.05$). B00: beer without hop replacement; B25: beer with 25% hop replacement; B50: beer with 50% hop replacement; B75: beer with 75% hop replacement.

The sensory analysis and purchase intention of beers are presented in Table 5. No significant difference ($p > 0.05$) was found for colour attributes in the beers. The beer with 25% hop replacement does not show significant differences compared to the control beer (B00) for all attributes. Bitterness intensity showed significant differences ($p < 0.05$) between the control beer (B00), B50, and B75. The bitterness (IBU) of the beers decreased with increased hop replacement (Table 3). The IBU of gervão-roxo is lower than that of hops (Table 2). However, polyphenols can impact the sensory bitterness intensity and bitterness units (IBU) of a beer (HAHN et al., 2018), which is in line with previous studies (LAZZARI et al., 2022; SCHUINA et al., 2020). The bitterness intensity of B00 and B25 is close to “slightly intense” and “moderately intense” and shows a higher purchase intention (Table 5). As previously mentioned, the consumers in this research prefer traditional/craft beers with low bitterness (Fig. 1a), which explains why B50 and B75 have the lowest purchase intention due to their greater intensity of bitterness. Phenolic acids in the composition of gervão-roxo in higher quantities may cause astringency, a quality that was not appreciated in beverages.

Principal component analysis

Principal component analysis (PCA) is used to graphically represent the relationship between variables (chemical composition and sensory attributes) for beers. The results are shown in Fig. 2. PCA explained 88.44% of the variables across two axes, PC1 (72.23%) and PC2 (16.21%). B00 and B25 are both positioned on the right side of PC1. The attributes associated with these beers align with their sensory characteristics (Table 5). Both B00 and B25 received higher ratings for sensory attributes (colour, aroma, flavour, overall acceptance), and the bitterness intensity for both was low, matching consumer preferences (Fig. 1a). On the other hand, B50 and B75 were positioned on the opposite side, showing an inverse relationship with consumer preferences.

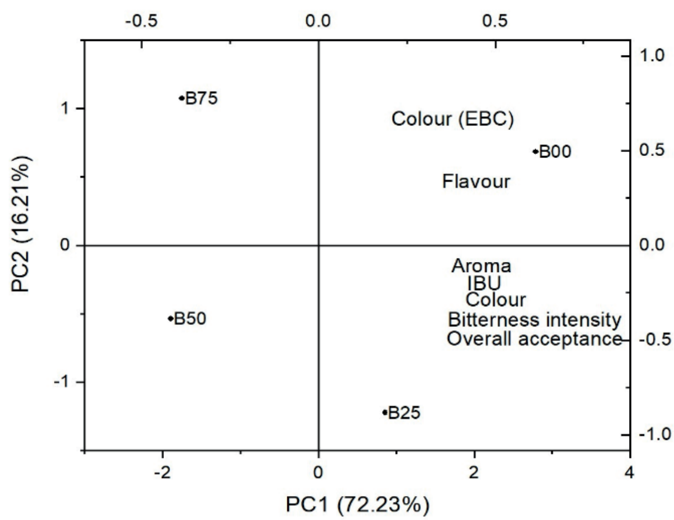

Figure 2. Principal component analysis (PCA) of beer sensory characteristics and chemical composition. IBU, International bitterness units; EBC, European Brewing Convention. B00: beer without hop replacement; B25: beer with 25% hop replacement; B50: beer with 50% hop replacement; B75: beer with 75% hop replacement.

CONCLUSIONS

Differences in the chemical composition and bioactive compounds between gervão-roxo and hops were observed, and these differences were related to the method of obtaining the raw material (bitter ingredient). Beers made with gervão-roxo as a bitter ingredient exhibited similar physicochemical characteristics and bioactive compounds to those of the standard sample (produced with hops), differing only in terms of bitterness, which can be attributed to intrinsic factors of the raw material. The results obtained, along with the sensory acceptance data, indicate that gervão-roxo can be effectively used as a bitter ingredient in beer production when combined with hops at a concentration of 25%.

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