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RUBIM (*Leonurus sibiricus*) e MASTRUZ (*Chenopodium ambrosioides* L.) COMO INGREDIENTES NA PRODUÇÃO DE CERVEJA ARTESANAL

ANDERSON LAZZARI

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BIOGRAFIA

Anderson Lazzari nascido em 29 de abril de 1993 na cidade de Palotina, Paraná, Brasil. Filho de Silvio Luiz Lazzari e Sirlei Eunice Pandolfo Lazzari. Possui graduação em Engenharia de Alimentos pela Universidade Tecnológica Federal do Paraná – Campus de Campo Mourão em 2017. Ingressou no Programa de Pós-Graduação em Ciências de Alimentos em abril de 2019, com defesa da dissertação em junho de 2021. Tem experiência nas áreas de Ciência e Tecnologia de Alimentos atuando na área de ciência e tecnologia de produtos agropecuários.

Dedico

Aos meus pais, que são meu porto seguro.

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APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de dois artigos científicos:

1 Anderson Lazzari; Heloisa Dias Barbosa; Evandro Ribeiro Machado; Lucas Henrique Maldonado da Silva; Fernando Antônio Anjo; Francielle Sato, Cássia Inês Lourenzi Franco Rosa; Paula Toshimi Matumoto Pintro. Effect of brewing process in bioactive compounds and antioxidant activity of beers made with medicinal plants. Food Research International

2 Anderson Lazzari; Heloisa Dias Barbosa; Evandro Ribeiro Machado Filho; Ana Paula Dada; Bianka Rocha Saraiva; Paula Toshimi Matumoto Pintro. Inlfuence of gender in acceptability of beers made with medicinal plants. International Journal of Gastronomy and Food Science.

GENERAL ABSTRACT

INTRODUCTION. Beer is the most consumed alcoholic beverage in the world. The principal's ingredients used for brewing process is malt and hops (*Humulus lupulus*). Hops were used as preservative agent for centuries, became one of principal ingredient due to their pleasant aroma. A growing segment in beverage industry is craft beer, due to an increase in consumption and interest in craft and special beer by consumers. Craft beers has a main characteristic the introduction of spices that insure new aromas and flavors for beverages. Different types of special beers were produced in world, differing on composition of raw and flavorings materials. Medicinal plants are effective as food additives, have in their composition phenolic compounds, such as terpenes responsible for aroma in beers and flavonoids usually responsible for color, taste, lipid oxidation prevention, vitamins, and enzymes protection. Rubim (*Leonurus sibiricus*) and Mastruz (*Chenopodium ambrosioides* L.) are medicinal plants rich in terpenes, flavonoids and phenolic compounds which are associated with bitterness in plants and teas. These medicinal plants can be new ingredients for manufacturing, introducing new flavors for beverages. Rubim and Mastruz might be a potentially hops replacement, improving bioactive compounds, antioxidant activity of beers, and can be able to please a greater number of consumers.

AIMS. The objective of this study was characterized physicochemical properties of medicinal plants and produce beers with hops replacement following the proportion: 25%, 50%, 75% and 100%. Evaluate physical-chemical properties, bioactive compounds, antioxidant activity and acceptability by consumers of beers made with medicinal plants.

MATERIAL AND METHODS. Rubim and Mastruz were obtained from southwest of Mato Grosso do Sul (22º 17' 23" S, 53º 16' 49" W), Brazil, sanitized in sodium hypochlorite, washed in distilled water, dried in oven with air circulation and ground at 60 mesh. Medicinal plants were analyzed for moisture, crude fiber, crude protein, ash, total fat, and total carbohydrate. The bioactive compounds (total phenolic content (TPC) and total flavonoid content (TFC)) and antioxidant activity (DPPH and ABTS assay), and FTIR (Fourier Transform Infrared) was performed for medicinal plants. Beers were produced with hop bitterness replacement based on International Bitterness Units (IBU) of medicinal plants. The proportion of replacement was: 25%, 50%, 75% and 100%. Beers with Rubim were nominated of BR25, BR50, BR75 and BR100. Beers with Mastruz of BM25, BM50, BM75 and BM100. Beer only hop was brewed and nominated beer standard (BS). Medicinal plants not mixed each other. Bioactive compounds (TPC and TFC), antioxidant activity (DPPH and ABTS assay), and IBU of wort before fermentation (WBF) and beers were analyzed. Acceptability of beers made with medicinal plants were evaluated by sensory analyzes and purchase intention.

RESULTS AND DISCUSSION. The medicinal plants presented higher protein content than hops, and similarities between medicinal plants and hops were observed by FTIR spectrums. Hop replacement by Rubim and Mastruz to brewing did not have a significant difference in total soluble solids and alcohol by volume. Beer with hop replacement by 100% Rubim showed significant difference in pH and acidity, BR50 presented significant difference in color (EBC). Beer with hop replacement by 25% Rubim (BR25) presented higher TPC than beer standard (BS). Beer with hop replacement by 100% Mastruz (BM100) presented higher TPC and TFC than BS. Antioxidant activity of BR100 was higher than BS for DPPH assay. BM100 in both assays presented higher activity than BS. BM100 presented an increased in TPC from WBF for beer. Losses in TPC, DPPH and ABTS assay were observed between wort before fermentation (WBF) and beers. Correlations between bioactive compounds and antioxidant activity were found by Pearson Correlations. Replacement of hop decreased IBU with increased of replacement by medicinal plants. Gender influenced in acceptability of beers by sensorial analyzes. Women seek new flavors in beer, and are more tasters than men, which are more traditional and influential by friends. Beers with hop replacement were accepted by consumers. BR100 was less accept beer.

CONCLUSIONS. Hops replacement by medicinal plants improved bioactive compounds and antioxidant activity of beers. International Bitterness Units (IBU) of beers was affected by hop replacement, yet bitterness intensity was felts by consumers sensorily. The hop replacement with 100% Mastruz pleasured consumers, especially women, which demonstrate these medicinal plants can be hop replacement, and that new ingredients will help craft beer industries in produce of new beverages.

Key words: Plant beer; medicinal plants; antioxidant capacity; gender preferences.

RESUMO GERAL

INTRODUÇÃO. A cerveja é a bebida alcoólica mais consumida no mundo. Os principais ingredientes na produção de cerveja são malte e lúpulo (*Humulus lupulus*). O lúpulo foi utilizado como conservante durante séculos, tornou-se um dos ingredientes principais devido ao seu aroma agradável. Um segmento crescente na indústria de bebidas é a cerveja artesanal, devido ao aumento do consumo e ao interesse por cervejas artesanais e especiais por parte dos consumidores. A cerveja artesanal tem como principal característica a introdução de especiarias que garantem novos aromas e sabores às bebidas. Diferentes tipos de cervejas especiais foram produzidos no mundo, diferindo na composição da matéria-prima e materiais aromatizantes. As plantas medicinais são eficazes como aditivos alimentares, possuem em sua composição compostos fenólicos, como os terpenos que são responsáveis pelo aroma em cervejas e os flavonoides que geralmente são responsáveis pela cor, sabor, prevenção da oxidação lipídica, vitaminas e proteção enzimática. Rubim (*Leonurus sibiricus*) e Mastruz (*Chenopodium ambrosioides* L.) são plantas medicinais ricas em terpenos, flavonoides compostos fenólicos que são associados ao amargor em plantas e chás. Essas plantas medicinais podem ser novos ingredientes para a fabricação, introduzindo novos sabores para as bebidas. Rubim e Mastruz podem ser potenciais substitutos do lúpulo, melhorando os compostos bioativos, a atividade antioxidante das cervejas, podendo agradar a um maior número de consumidores.

OBJETIVO. O objetivo deste estudo foi caracterizar as propriedades físico-químicas das plantas medicinais e produzir cervejas com substituição ao lúpulo seguindo a proporção: 25%, 50%, 75% e 100%. Avaliar as propriedades físico-químicas, compostos bioativos, atividade antioxidante e aceitabilidade por consumidores das cervejas produzidas com plantas medicinas.

MATERIAL E MÉTODOS. Rubim e Mastruz foram obtidos do sudoeste de Mato Grosso do Sul (22º 17’ 23” S, 53º 16’ 49” O), Brasil, higienizadas em hipoclorito de sódio, lavadas em água destilada, secadas em estufa de circulação de ar e trituradas a 60 mesh. As plantas medicinais foram analisadas quanto à umidade, fibra bruta, proteína bruta, cinzas, gordura total e carboidratos totais. Os compostos bioativos (conteúdo fenólico total (TPC) e conteúdo total de flavonoides (TFC)) e atividade antioxidante (ensaio DPPH e ABTS) e FTIR (Infravermelho com Transformada de Fourier) foram realizadas para as plantas medicinais. As cervejas foram produzidas com substituição do amargor do lúpulo com base em Unidades Internacionais de Amargor (IBU) das plantas medicinais. A proporção de substituição foi: 25%, 50%, 75% e 100%. As cervejas com Rubim foram nomeadas de BR25, BR50, BR75, BR100. As cervejas com Mastruz de BM25, BM50, BM75 e BM100. Foram produzidas uma cerveja só com lúpulo e nomeada de cerveja padrão (BS). As plantas medicinais não foram misturadas. Os compostos bioativos (TPC e TFC), atividade antioxidante (ensaio DPPH e ABTS), e IBU do mosto antes da fermentação (WBF) e cervejas foram analisados. A aceitabilidade das cervejas com plantas medicinais

foi avaliada por análises sensoriais e intenção de compra.

RESULTADOS E DISCUSSÃO. As plantas medicinais apresentaram maior teor de proteína que o lúpulo, e semelhanças entre as plantas medicinais e o lúpulo foram observadas por espectros de FTIR. A substituição do lúpulo por Rubim e Mastruz para a fabricação de cerveja não teve diferença significativa nos sólidos solúveis totais e teor alcoólico. Cerveja com substituição de lúpulo por Rubim 100% apresentou diferença significativa no pH e acidez, BR50 apresentou diferença significativa na cor (EBC). Cerveja com substituição de lúpulo por 25% Rubim (BR25) apresentou maior TPC que a cerveja padrão (BS). Cerveja com substituição do lúpulo por Mastruz 100% (BM100) apresentou maior TPC e TFC que BS. A atividade antioxidante de BR100 foi maior que BS para o ensaio DPPH. BM100 em ambos os ensaios apresentou maior atividade que BS. BM100 apresentou um aumento em TPC do WBF para a cerveja. Perdas em TPC, ensaio DPPH e ABTS foram observadas entre o mosto antes da fermentação (WBF) e as cervejas. Correlações entre compostos bioativos e atividade antioxidante foram encontradas pelas correlações de Pearson. As substituições do lúpulo diminuíram o IBU com o aumento das substituições por plantas medicinais. O gênero influenciou na aceitabilidade das cervejas por análises sensoriais. As mulheres buscam novos sabores na cerveja, e são mais degustadoras do que os homens, que são mais tradicionais e influenciáveis pelos amigos.

CONCLUSÃO. A substituição do lúpulo por plantas medicinais melhorou os compostos bioativos e atividade antioxidante das cervejas. Unidades Internacionais de Amargor (IBU) das cervejas foi afetado pela substituição do lúpulo, no entanto, a intensidade do amargor foi sentida pelos consumidores sensorialmente. A substituição do lúpulo com 100% Mastruz agradou os consumidores, especialmente mulheres, demonstrando que essas plantas medicinais podem ser substitutos do lúpulo, e que esses novos ingredientes ajudarão a indústria de cervejas artesanais na produção de novas bebidas.

Palavras chaves: Cerveja com planta; plantas medicinais; capacidade antioxidante; preferências de gênero**.**

ARTICLE 1

Effect of brewing process in bioactive compounds and antioxidant activity of beers made with medicinal plants

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Abstract

Replacement of hops by medicinal plants and step of beer process can influence in bioactive compounds content, antioxidant activity and International Bitterness Units (IBU) in beers. Rubim (*Leonurus sibiricus*) and Mastruz (*Chenopodium ambrosioides* L.) has important nutrients, such as proteins, carbohydrates, and phytochemical compounds. Beers with hops replacement were brewed following the proportion: 25%, 50%, 75% and 100%. Medicinal plants not mixed each other. Bioactive compounds, antioxidant activity and IBU were analyzed in wort before fermentation (WBF) and beer. Beer with hop replacement by 25% Rubim and with 100% of Mastruz presented higher total phenolic compounds (TPC). Hops replacement with 100% Rubim and Mastruz presented higher antioxidant activity by DPPH. However, ABTS was lowest for hop replacement by 100% Rubim. IBU decreased with hop replacement by Rubim and Mastruz. Beers with medicinal plants showed 20% overall loss in TPC, excepted BM100 which increase. Beers increased TFC, excepted BR25 and BM25. Positive correlations between TPC and ABTS, and between TFC and ABTS in beers with Rubim, between TPC and antioxidant activity (DPPH and ABTS), TFC and ABTS in beers with Mastruz are found. Principal component analysis (PCA) was performed to understand interrelationships among measured bioactive compounds and antioxidant activity.

Keywords: Medicinal plants; Brewing; Antioxidant capacity; Bitterness

1. Introduction

Beer is an alcoholic beverage consumed worldwide. Rich in carbohydrates, amino acids, minerals, and phenolic compounds (Ducruet et al., 2017). The importance of beverages containing polyphenols such as teas, coffees, fruit juices, wines and beers are well recognized (Leitão et al., 2011). Correlations between level of polyphenols and antioxidant activity are reported in beers (Gorinstein et al., 2007; Gorjavonic et al., 2010).

Phenolic compounds characterization in raw material and what happens to these compounds in beer process are important, since polyphenols influence the formation of beer haze, color, bitterness, astringency, foam stability and redox state (Šibalić et al., 2021). General chemical composition of malt wort depends on temperature profile during mashing (Zhao & Zhao, 2012). During boiling and fermentation occurs polymerization of phenolics compounds into polyphenols, contributing to beer flavor stability and have a role in aging of beer (Wannenmacher et al., 2018).

Medicinal plants are commonly consumed by several biological activities and numerous health benefits, contain in chemical composition different compounds, such as lipids, flavonoids, and phenolic acids (McKav and Blumberg, 2006; Chan et al., 2012). Beers made with medicinal plants has an increase in concentration of bioactive compounds, due to presence of secondary metabolites, such as alkaloids, saponins, tannins, which hold different biological activities, ensuring fluid food safety, shelf life and quality of fluid food (Sharma et al., 2016; Ducruet et al., 2017). Polyphenols as antioxidant are important for health of consumers (Mehra et al., 2020). Antioxidant activity of plants are attributed to phenolic compounds, quenching free radicals, or inhibiting activity of free-radical-generating enzymes, acting as reducing agents or metal chelators (Santhakumar et al., 2013). Preventing oxidation of other molecules presented in beer, flavonoids such as flavan-3-ols and their condensed products have capacity to improve oxidative stability on beer (Aron & Shellhammer, 2010). Flavonoids are natural bioactive compounds found in plant food, leaves, seed, roots, and stems (Kunisuke et al., 2010).

Bitter tastes and foam to beer are supplied especially by alkaloids, tannins, saponins, and numerous other phytochemicals are responsible for countless proprieties which hops supply for beer (Okafor et al., 2020). Hop phytochemicals are found in medicinal plants and replacement of hop proportion by medicinal plants and the evaluate their capacity of act as hop phytochemicals are the aim of this study. Two medicinal plants were chosen based on phytochemicals composition. Mastruz (*Chenopodium ambrosioides* L.) and Rubim (*Leonurus sibiricus*) are medicinal plants with inflammatory, antioxidant, antimicrobial proprieties (Sayed et al., 2016; Zohra et al., 2018). Flavonoids, alkaloids, tannins, saponins, and phenolic compounds (Sayed et al., 2016; Zohra et al., 2018) are found in their phytochemical's composition. This study is aimed at investigating replacement of hop by medicinal plants in beer brewing based in physical-chemical properties, evaluated bioactive compounds and antioxidant activity of wort before fermentation and beers made with hops replacement.

2. Material and methods

2.1 Materials

Rubim and Mastruz were obtained from southwest of Mato Grosso do Sul, Brazil (22º 17' 23" S, 53º 16' 49" W). The tradition hop was obtained from a traditional Pilsner-type beer production from Industrial Norte Paranaense de Bebidas (INBEB; Londrina, PR, Brazil), and stored at 10ºC until analyses.

Folin-Ciocalteau, gallic acid, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulphate, sodium carbonate and 2,2,4-trimethylpentane were from Sigma-Aldrich (St. Louis, Missouri, USA). Aluminum chloride, HCl were of analytical grade.

2.2 Preparation of the raw material

Rubim and Mastruz were washed in running water, sanitized in sodium hypochlorite solution (200ppm/10min) and washed with distilled water. After, dried at 55ºC for 48h in oven with air circulation. Dried plants were ground, sieved to 60 mesh and stored under refrigeration, protected from light.

2.3 Chemical composition and caloric of medicinal plants and hop

Chemical composition was analyzed for moisture (925.09; AOAC, 2005), crude fiber (Ba 6a-05; AOCS, 1996), crude protein (Kjedahl method), ash (923.03); (AOAC, 2005) and total fat (Bligh & Dyer, 1959). Total carbohydrate was determined by difference. Results were expressed on dry matter basis. The caloric value (kcal $100g^{-1}$) was estimated according to Li et al., 2014, as follow:

Caloric value = (4 x %protein) + (4 x %carbohydrate) + (9 x %fat)

2.4 FTIR-ATR of medicinal plants and hop

Medicinal plants and hop were analyzed using an FTIR apparatus (Vertex 70v; Bruker, Ettlingen, Germany). Samples were positioned and primed on diamond crystal of attenuated total reflectance device so that all diamond was in contact with sample during measurements. A total of 128 scans were obtained per spectrum, at 4 cm^{-1} resolutions, from 400 to 400 cm^{-1} (Ubaldini et al., 2012).

2.5 Bioactive compounds and antioxidant activity

2.5.1 Bioactive compounds extract

Bioactive compounds extract (BCE) of medicinal plants and hop (1:50 w/v) were homogenized (10 min) with deionized water. After centrifugation at 963 x ***g*** for 10 min, the supernatant was collected and analyzed. Wort before fermentation (WBF) and beer were centrifuged at 3000 rpm for 15 min to degasify the samples.

2.5.2 Total phenolic compounds (TPC)

TPC was determined according to Singleton & Rossi (1965) with modifications. BCE, WBF or beer (125 µL) was mixed with 125 µL of Folin–Ciocalteau reagent (1:1 deionized water) and 2250 µL of sodium carbonate (28 g L^{-1}), homogenized and incubated in dark at 25 °C for 30 min. Absorbance was measured at 725 nm, and results to plants were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample. WBF and beer as milligrams of gallic acid equivalents (GAE) per liter.

2.5.3 Total flavonoid content (TFC)

TFC was determined according to Buriol et al., 2009. BCE, WBF or beer (300 µL) was mixed with 150 µL of $AlCl_3$ and 2550 µL of methanol. Samples was incubated for 30 min in dark and measured by spectrophotometer at 425 nm. Results of plants were expressed as quercetin equivalent (mg QE/g sample). WBF and beer per liter (mg QE/L).

2.5.4 DPPH and ABTS assay

DPPH assay was undertaken according to Li et al. (2009) with modifications. BCE, WBF or beer (150 µL) was mixed with 2.85 mL of DPPH solution (60 µM). After incubation in dark for 30 min, the absorbance was measured at 515 nm.

ABTS free radical was generated by oxidation of ABTS (7 mM) with potassium persulfate (2.45 mM). The reaction mixture was left to stand at room temperature for 12h in dark prior to use, and the $ABTS^{+\cdot}$ solution was diluted with ethanol until an absorbance of 0.700 ± 0.020 at 734 nm was achieved. ABTS assay was determined according to Re et al. (2011) with modifications. $ABTS^{+\cdot}$ solution (1960 µL) was mixed with BCE, WBF or beer (40 µL), and the absorbance was measured at 734 nm after 6 min in dark. Antioxidant activities were calculated using the following equation:

Antioxidant activity (%) = (1- ($A_{sample\ t}$/$A_{sample\ t=0}$)) x 100

where: $A_{sample\ t}$ is samples absorbance at 30 min (DPPH) and 6 min (ABTS), and $A_{sample\ t=0}$ is samples absorbance at time zero.

2.6 Brewing technology

Beers were produced by modified ale-type beer brewing method, with replacement of hop by medicinal plants (Rubim and Mastruz). Wort was prepared, using commercially Pilsen malt according to following mashing programmer: 30 min at 44ºC, 20 min at 52ºC, and 30 min at 70ºC. The mash was then heated to 76ºC and filtered to yield wort, which was kept boiling for 60 min at 98ºC. Bittering agents (hops, Rubim or Mastruz) were added at beginning of boiling. The hop bitterness replacement was based on global bitterness (IBU) of hop, Rubim and Mastruz: 40, 12 and 25, respectively. Medicinal plants proportion were 25%, 50%, 75%, and 100% from hop bitterness. Plants has not mixed each other. Nine beer formulations were produced, with the beer standard. After mashing, wort was cooling, filtrated, and collected to analyzes before fermentation (WBF). US-05 yeast was added, and fermentation was carried out at 20ºC for 7 days. Maturation was held at 3ºC for two weeks. Beers were stored at amber glass bottles, sugar (3g/L) was added to promote carbonation, at 23ºC for a week. Beers have been pasteurized (65ºC at 10 min). Beers were collected and then analyzed for bioactive compounds, antioxidant activity and IBU.

2.6.1 Analyzes of International Bitterness Units (IBU)

Bitterness level of WBF and beers were analyzed. WBF or beers (2 mL) degassed were transferred to Falcon tubes (15 mL), and 200 µL of hydrochloric acid solution (3 N HCl) and 4 mL 2,2,4-triethylpentane were added to the tube (Analytica-EBC, 2010). The tubes were shaken for 15 min, and centrifuged (3000 rpm, 10 min). A sample for analyzes was collected from the supernatant and determined spectrophotometrically by absorbance at 275 nm. IBU was calculated by: IBU = 50*A (A is absorbance at 275 nm).

2.7 Statistical analyzes

The plants and hop data were evaluated by analyzes of variance using the general linear model procedure in SPSS (v. 19.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) for Windows. Data are presented as the mean and standard deviation. Differences were considered significant at $P < 0.05$, Tukey's test was performed. Linear dependence was judged by Pearson correlation coefficients (R). Principal components analyze (PCA) was performed for graphical illustration of relations among characteristics performed in Origin 2018. Brewing process was carried out five times. All measurements were performed in triplicate.

3. Results and discussion

3.1 Brewing raw material properties

Physical-chemical properties, bioactive compounds and antioxidant activity of medicinal plants and hops are presented in Table 1. Hops (*Humulus lupulus* L.) is an industrial and medicinal plant of great importance in brewing industry due to organoleptic characteristics. These characteristics are derived of bitter organic acids, essential oils, resins, and polyphenol compounds (Keskin et al., 2019), similar organoleptic characteristics are presented in Rubim and Mastruz, makes interesting study for replacement of hops in beer production.

Beer quality and flavor depends particularly on mineral contents of brewing water and is important for the brewing process (Montanari et al., 2019). Wort boiling decreased mineral concentration because of metal binding to precipitated material (e.g., salts with acidic groups of proteins and trub), with hops addition, minerals are restored (Montanari et al., 2019). Hops contribution for minerals in beer is negligible due to small quantities used, especially Mastruz may be a good source of minerals for brewing process due to ash content. Rubim presented significant difference in total carbohydrate content of Mastruz and hop. Carbohydrates are essential for fermentation in beer, as fermentable sugars (monosaccharides and oligosaccharides) contribute to sweetness and complex carbohydrates contribute to 'body' and mouthfeel of beer (Li et al., 2020).

Crude protein content of Mastruz and Rubim are higher than hop. Protein influences the whole brewing process. As enzymes, degrade starch, β-glucans, and proteins. Foam stability, mouthfeel and flavor stability in beer depends on protein-protein linkages. Such as amino acids, peptides, and salt ammoniac are important nitrogen sources to yeast (Steiner et al., 2011). Interaction's protein-polyphenol in brewing process is important for haze in beer. The haze is a desired quality attribute in craft beers, lager and pilsner type beers of bottom-fermented, phenolic flavor is recognized as off-flavor (Wannenmacher et al., 2018).

The medicinal plants presented higher total flavonoids compounds than hops, flavonoids may be potent antioxidants, depend on the number of OH substituents. The concentration and free radical source can influence if flavonoids function like an anti- or prooxidant (Wannenmacher et al., 2018). Plants showed lower total phenolic compounds (TPC) and antioxidant activity

(DPPH and ABTS) than hops, chemical constituents in plants are influenced by plant species, plant age, geographical location, soil nutrients, and climate conditions (Ray et al., 2013), that may explain the content of bioactive compounds in Mastruz and Rubim.

3.2 FTIR-ATR of medicinal plants and hops

FTIR-ATR spectrum of medicinal plants and hops are showed in Figure 1. Activity of specific antioxidant is closely relative on their molecular structure, specifically, number and position of OH groups are important (Masek et al., 2014). The peaks at 2960 cm^{-1} indicated strong bands of saturated -CH stretching (Agatonovic-Kustrin et al., 2020). Peaks with centers at approximately 1733 cm^{-1} corresponds to C=O stretching vibration (Yu, 2004). Dienes, trienes and α, β-unsaturated carbonyl are presented in 1609 cm^{-1} (Agatonovic-Kustrin et al., 2020). The bands between 1350 and 1600 cm^{-1} confirmed aromatic structures by C=C skeleton vibrations (Masek et al., 2014). Bands between range 1100-1300 cm^{-1} can indicate that carbonyl group is derived from carboxyl groups or may be present in phenyl compounds (Masek et al., 2014). The band 1025 cm^{-1} corresponding to C-H bend of substituted aromatic compounds (Johson et al., 2020). An intense peak at 828 cm^{-1} is linked with terpenoids (Agatonovic-Kustrin et al., 2020). The band in 780 cm^{-1} was associated with –CH in substituted benzenes linked to carboxylate groups (Yu, 2004). FTIR analyzes revealed presence of alkaloids, polyphenols and flavonoids, and terpenes due to N-H stretching, O-H stretching, C-H group, respectively for medicinal plants and hops.

3.3 Bioactive compounds, antioxidant activity and International Bitterness Units (IBU) of WBF and beers

Bioactive compounds of WBF and beers are presented in Figure 2. Differences were observed in WBF with hops replacement by Rubim or Mastruz. WBF of BR25 presented higher TPC than WBF of BS. BR50 and BR100 (WBF) presented lowest TPC. In beer, differences were observed for all treatments (Figure 2a) and losses in polyphenol compounds were observed in all beers with Rubim (Table 2a). The highest loss was presented in BR75, followed by BR100 and BS. WBF with replacement of hops by Mastruz presented differences for TPC (Figure 2b). Highest TPC was observed in BS (WBF) followed by BM100 (WBF). Loss in TPC was observed in beers with Mastruz, except to BM100 that presented an increase (8.5%) (Table 2b). Overall, these finds in phenolic compounds after mashing was expected, since mashing occurs under conditions adequate for enzyme action, and probably leads to release of phenolics compounds bound (Zhao, 2015). The type of mashing had a strong impact on phenolic content.

Worts produced with decoction mashing showed higher TPC than worts produced with infusion mashing (Zhao, 2015). In this study, decoction mashing was performed which may be linked with high TPC in WBF.

Several steps are carried out until final product, such as fermentation, maturation, filtration of wort and pasteurization of beer. Losses in phenolic compounds might be due to precipitation of tannins and non-tannin phenolics from worts and beers absorption by yeast during fermentation (Szwajgier, 2009). Beer filtration, removes yeast cells, causing loss of effect on the redox potential due to NADH and NAD^+. Permanent and chill-haze are removed, inducing to loss of polyphenols (Pascoe et al., 2003). BM100 was only beer with increased in TPC. Increase in phenolics during pasteurization is mentioned in previously studies, however the cause is not known (Pascoe et al., 2003).

Total flavonoid compounds are presented in Figure 2c and 2d. Curiously, beers made with 25% of replacement of hops by Rubim and Mastruz presented decreased in TFC (Table 2a, 2b). Even decreased in TPC, the TFC increased or remained stable during brewing process (Table 2a, 2b). Stability of flavonoid compounds with decreased in phenolic compounds was attributed to the oxidation of phenolic compounds by free radicals and polymerization with proteins, reported in previously studies in beer enriched with eggplant peel extract (Horincar et al., 2020).

The radical system used for determinate antioxidant activity may influence experimental results, two or more radical systems are required to investigate radical scavenging capacities of antioxidant matrices (Yu & Zhu, 2005). DPPH and ABTS are partly different. DPPH radical reacts with polyphenols, but not with phenolic acids and sugars, ABTS assay react with more compounds, which influences in stability, can lead to unbiased results (Mareček et al., 2017). Antioxidant activity results of WBF and beers are presented in Figure 3. Differences were observed between WBF made with replacement of hops by Rubim and WBF of beer standard (BS) for DPPH assay (Figure 3a). A decreased in DPPH value was observed for all beers, BR75 was the beer with higher loss (Table 3a). All WBF with replacement of hops by Mastruz (Figure 3b) presented difference of BS (WBF) and highest values for DPPH assay. Differences were observed between beer treatments, and a decreased in DPPH assay was observed for all beers with Mastruz. Higher losses were observed in BRM50 and BM75 (Table 3b).

ABTS assay presented difference between treatments in WBF with replacement of hops by Rubim or Mastruz (Figure 3c, 3d). BS and BR50 increased ABTS (Table 3a). All beers with Mastruz decreased ABTS content (Table 3b). Before fermentation (WBF), high antioxidant activity is expected, due to elevated temperatures during boiling process, Maillard reaction products with antioxidant activity was formed, attributed to increase in antioxidant activity

(Pascoe et al., 2003). The lowest values obtained in ABTS assay for beers with higher replacement of hops by Rubim or Mastruz (Figure 3c, 3d), can be influenced by the protein content (Table1). Overall, higher protein content decreases antioxidant activity (Mareček et al., 2017).

International Bitterness Units (IBU) of WBF and beers are presented in Figure 4. The boiling process produces compounds on the main source of bitterness in beers – iso-α-acids (isohumulones), from hop α-acids (humulones) (Oladokun et al., 2016). Bitter acids (IBU) are an analytical measure of the expected quantity in beer and gives an approximate value of iso-α-acids in milligram per liter of beer (Hough et al., 1982). A decrease in IBU was observed for beers with replacement of hops by Rubim and Mastruz (Figure 4a, 4b). IBU of Rubim or Mastruz are smaller than hops. Aromatic compounds with C=O and C-OH linkages, are presented at a higher frequency in hops (Figure 1), can be soft resins, linked with α-acids, while Rubim and Mastruz presented lowest frequency these compounds, and presented linkages attributed to polyphenols, which contributes more to flavor in beers. Bitterness from polyphenols compounds is not measured by IBU analyzes, yet this bitterness was felt sensory as reported on study of hops replacement by carqueja extracts, rich in polyphenols, higher carqueja concentrations resulted in higher bitterness intensity (Schuina et al., 2019).

3.4 Pearson correlation analyzes

Correlations between phenolic compounds and antioxidant activity of grain, vegetables and other botanical materials have been reported (Zhao et al. 2008; Croge et al., 2019). Beers made with replacement of hops by Rubim and Mastruz were used to analyzed correlations between antioxidant activity evaluation indices and TPC, and results were presented in Table 3. The highest correlation coefficient was found between TPC and ABTS radical cation scavenging activity in beer made with Rubim, and a negative correlation between TPC and DPPH radical scavenging activity (Table 4a). Rubim beers with lower losses in TPC presented less losses in ABTS content (Table 2a, Table 3a), and BR50 presented higher TFC (Figure 2c) with lower loss in ABTS, corroborating with Pearson correlation (Table 4a). Beers with Mastruz presented higher correlations between TPC and antioxidant activity for both assays' DPPH and ABTS scavenging activity (Table 4b). The poor correlation between TPC and DPPH for beers with Rubim in this study suggested that antioxidant activity of these beers might partly depend on functional groups and content of individual phenolic compounds. A significant correlation between TFC and ABTS scavenging activity was found for beers with Rubim (Table 4a), and between TFC and DPPH scavenging activity in beers with Mastruz (Table 4b). BM100 presented higher TPC and TFC (Figure 2b, 2d) and consequently lower losses in DPPH and ABTS assay (Table 3b), corroborating with Pearson correlation (Table 4b). Flavonoids have been reported like free radical scavengers, metal chelators and strong antioxidants (Kumar & Pandey, 2013). Enrichment in flavonoids observed in most of beers might also account, partly, for the higher antioxidant measures in most of beers. A strong negative correlation was found for beers with Mastruz between TFC and IBU (Table 4b), was observed that beers with the highest TFC had lowest IBU. Flavanols form protein-polyphenol complexes, which are removed by filtration (Oladokun et al., 2016), these complexes may contain substances responsible for bitterness of beer.

3.5 Multivariate data analyzes

Principal components analyze (PCA) was performed to understand interrelationships among the measured antioxidant activity evaluation indices, TPC, TFC and IBU of beers. Results of PCA for beers with Rubim are shown in Figure 5a. Two principal components, explaining 80.93% of the total data variances. PCA expressed correlations between antioxidant activity evaluation indices, TFC and TPC. The first principal component (P1) correlated with ABTS radical cation scavenging activity, TPC and TFC. The second principal component (P2) was related to TFC, IBU, ABTS and DPPH. BR75 and BR100 formed a group and are on the opposite side of bitterness (IBU). Figure 5b shows Principal component analyzes (PCA) for beers with Mastruz. Two principal components explaining 83.81% of the total data variances. The first principal component (P1) correlated with DPPH and ABTS scavenging activity, TPC and TFC. BS and BM25 formed a group and were aligned with compounds such as IBU in WBF and beer. BM100 are in the same side of TPC, TFC, DPPH and ABTS assay and opposite side of bitterness (IBU). Correlations for beers with Rubim and Mastruz are like the conclusion from the Pearson correlations analyzes. Results shows significant correlations between bioactive compounds and antioxidant, each medicinal plant with a particular correlation. Beers made with hops replacement are linked with higher TPC, TFC and antioxidant activities and beer standard with higher IBU.

4. Conclusion

The present study showed that medicinal plants had influences on bioactive compounds (TPC and TFC) and antioxidant activity (DPPH e ABTS). Results showed beer with hop presented significant losses in bioactive compounds and antioxidant activities even with their rich composition. Beers made with Rubim and Mastruz presented high bioactive compounds and antioxidant activities and showed be great hop replacements, even medicinal plants presented lower bioactive compounds and antioxidant activity content than hop, beers with hop replacement presented values higher or equal to BS. Correlations between antioxidant activity, bioactive compounds and beers with medicinal plants were revealed by Pearson Correlation analyzes and PCA. This study demonstrated that medicinal plants can be hop replacement in brewing beers, partial or total. These medicinal plants improved bioactive compounds, antioxidant activity, and beer flavor stability.

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Declaration of interest: none

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Figure caption

Figure 1. FTIR-ATR of hop and medicinal plants

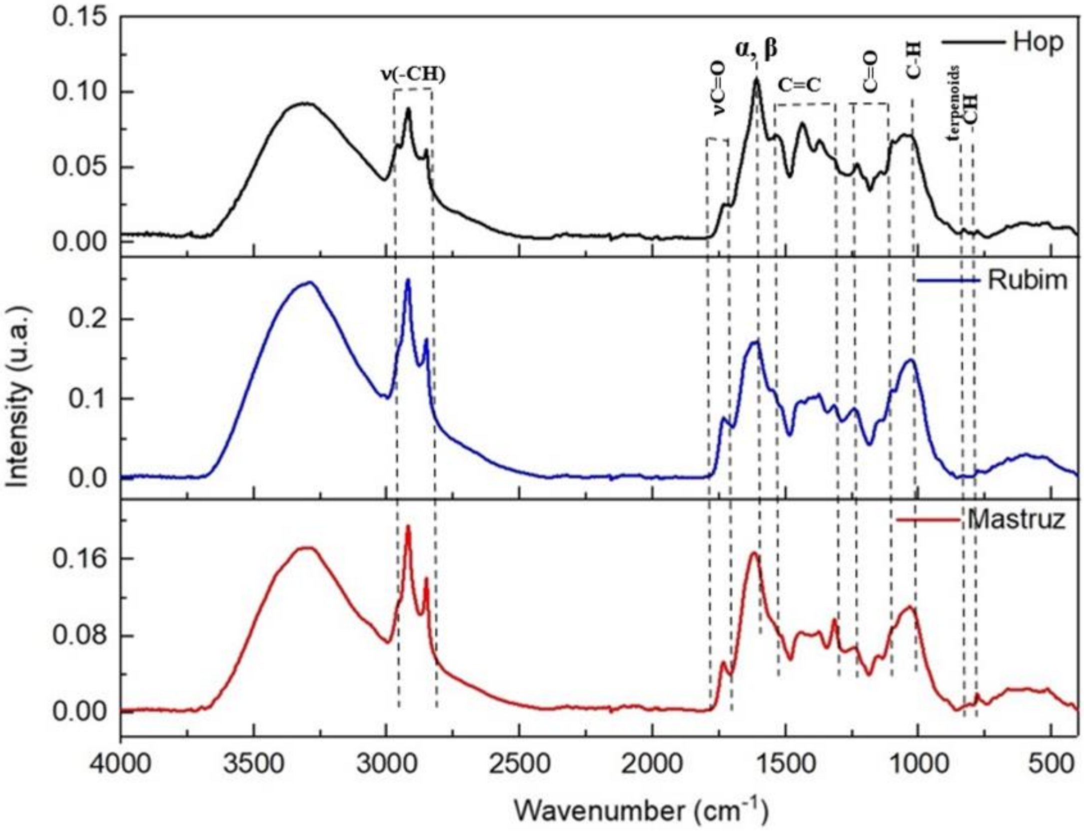
Figure 2. Total phenolic compounds of wort before fermentation (WBF) and beers with (a) Rubim and (b) Mastruz and total flavonoid compounds of beers with (c) Rubim and (d) Mastruz. Different letters indicate difference in the same process step. BS, beer standard; BR25, beer with hop replacement by 25% Rubim; BR50, beer with hop replacement by 50% Rubim; BR75, beer with hop replacement by 75% Rubim; BR100, beer with hop replacement by 100% Rubim; BM25, beer with hop replacement by 25% Mastruz; BM50, beer with hop replacement by 50% Mastruz; BM75, beer with hop replacement by 75% Mastruz; BM100, beer with hop replacement by 100% Mastruz. TPC, total phenolic compounds; GAE, gallic acid equivalent; TFC, total flavonoids compounds; QE, quercetin equivalent.

Figure 3. Free radical scavenging DPPH of wort before fermentation (WBF) and beers with (a) Rubim and (b) Mastruz and free radical scavenging ABTS of wort before Fermentation (WBF) and beers (c) Rubim and (d) Mastruz. Different letters indicate difference in the same process step. BS, beer standard; BR25, beer with hop replacement by 25% Rubim; BR50, beer with hop replacement by 50% Rubim; BR75, beer with hop replacement by 75% Rubim; BR100, beer with hop replacement by 100% Rubim; BM25, beer with hop replacement by 25% Mastruz; BM50, beer with hop replacement by 50% Mastruz; BM75, beer with hop replacement by 75% Mastruz; BM100, beer with hop replacement by 100% Mastruz.

Figure 4. International Bitterness Units (IBU) of wort before fermentation (WBF) and beers with (a) Rubim and (b) Mastruz. Different letters indicate difference in the same process step. BS, beer standard; BR25, beer with hop replacement by 25% Rubim; BR50, beer with hop replacement by 50% Rubim; BR75, beer with hop replacement by 75% Rubim; BR100, beer with hop replacement by 100% Rubim; BM25, beer with hop replacement by 25% Mastruz; BM50, beer with hop replacement by 50% Mastruz; BM75, beer with hop replacement by 75% Mastruz; BM100, beer with hop replacement by 100% Mastruz

Figure 5. Principal components analysis (PCA) of bioactive compounds, antioxidant activity and IBU of beers with (a) Rubim and (b) Mastruz. WBF: Wort before fermentation; B: beer.

Figure 1



Hop
Rubim
Mastruz
ν(-CH)
νC=O
α, β
C=C
C=O
C-H
terpenoids
-CH
Intensity (u.a.)
Wavenumber (cm-1)

Figure 2

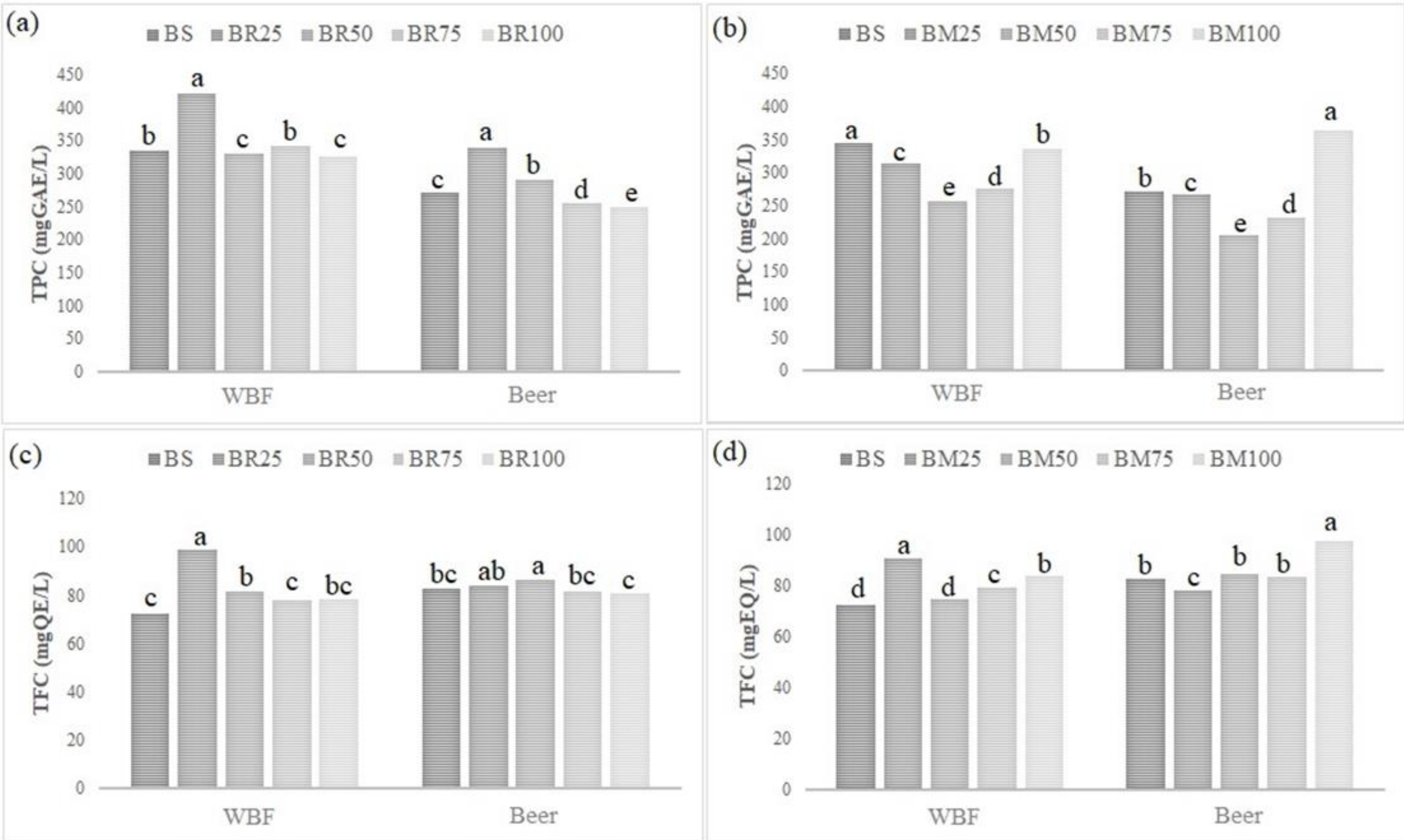

(a)
BS BR25 BR50 BR75 BR100
TPC (mgGAE/L)
WBF
Beer
(b)
BS BM25 BM50 BM75 BM100
TPC (mgGAE/L)
WBF
Beer
(c)
BS BR25 BR50 BR75 BR100
TFC (mgQE/L)
WBF
Beer
(d)
BS BM25 BM50 BM75 BM100
TFC (mgEQ/L)
WBF
Beer

Figure 3

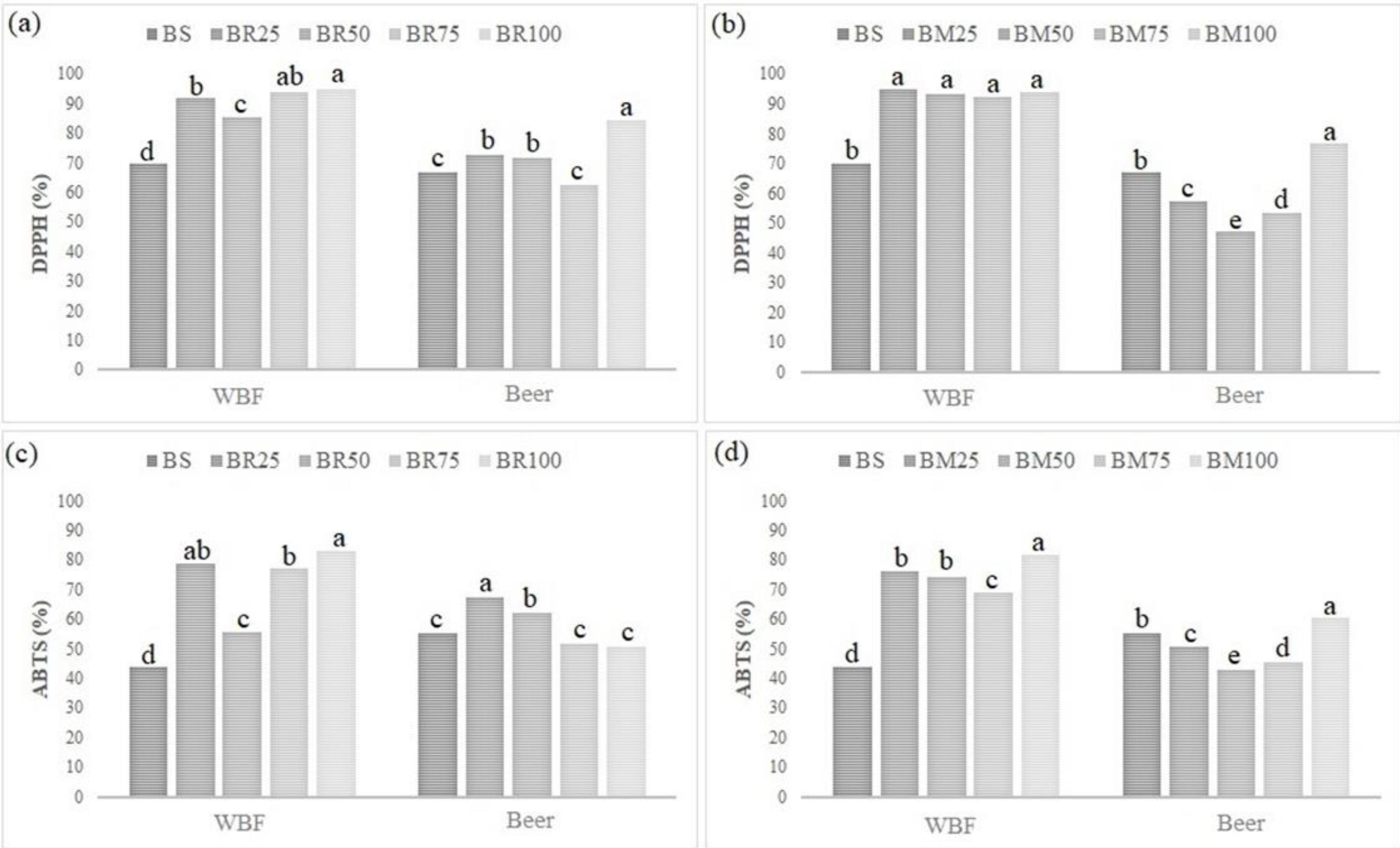


Figure 4

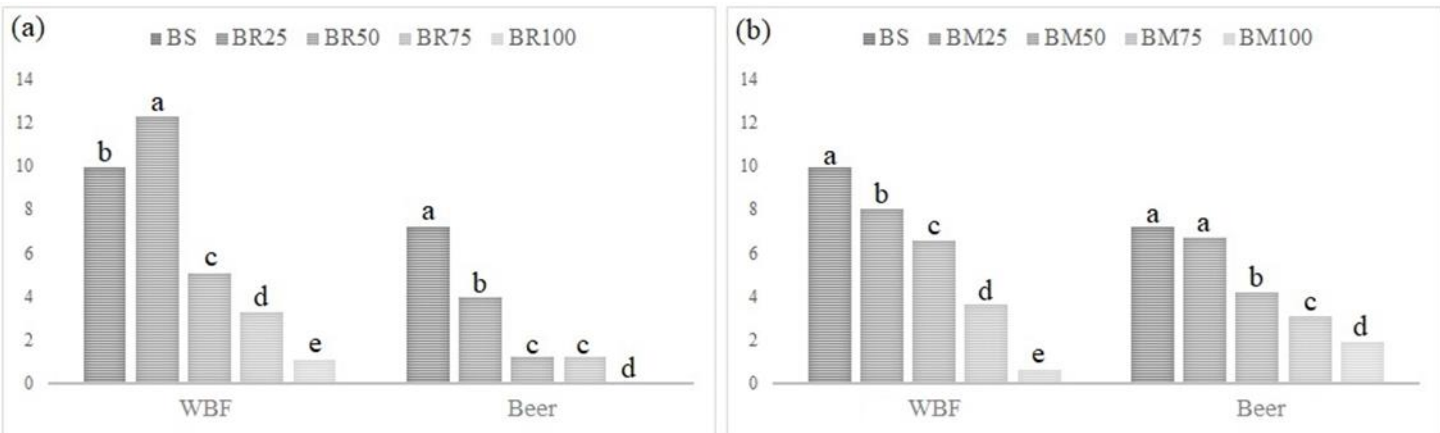


Figure 5

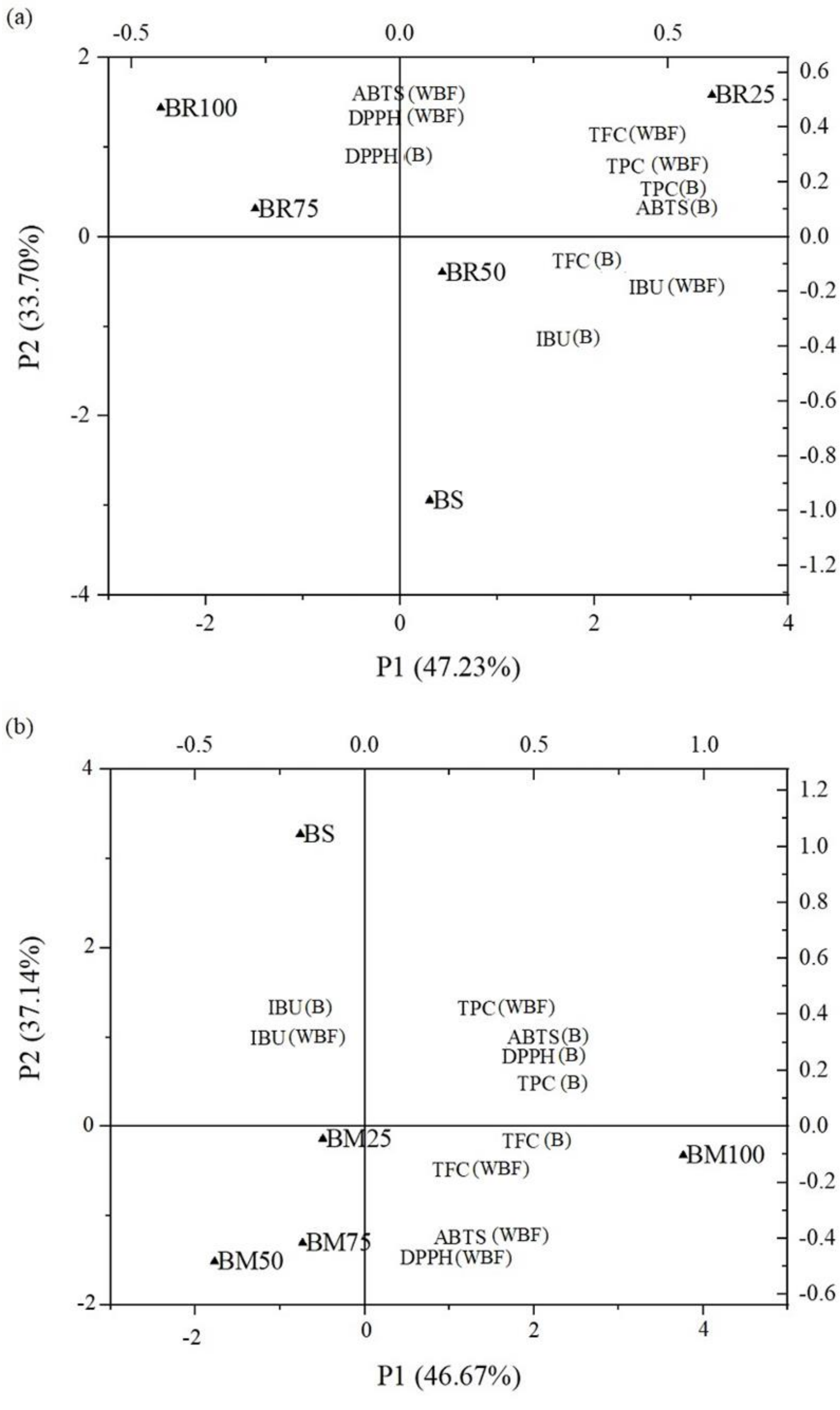

(a)
BR100
BR75
BR50
BS
BR25
ABTS (WBF)
DPPH (WBF)
DPPH (B)
TFC (WBF)
TPC (WBF)
TPC(B)
ABTS(B)
TFC (B)
IBU (WBF)
IBU(B)
P2 (33.70%)
P1 (47.23%)
(b)
BS
IBU(B)
IBU (WBF)
TPC (WBF)
ABTS(B)
DPPH (B)
TPC (B)
BM25
TFC (B)
TFC (WBF)
BM100
ABTS (WBF)
DPPH (WBF)
BM75
BM50
P2 (37.14%)
P1 (46.67%)

Table 1. Physical-chemical properties, bioactive compounds and antioxidant activity of medicinal plants and hop dried.

	Hop	Rubim	Mastruz
Physical-chemical properties			
Moisture (%)	10.21 ± 0.01^{a}	7.87 ± 0.20^{c}	9.71 ± 0.10^{b}
Crude protein (%)	14.58 ± 0.25^{c}	19.53 ± 0.11^{b}	21.13 ± 0.13^{a}
Fat (%)	15.56 ± 0.60^{a}	12.54 ± 0.36^{b}	7.96 ± 0.15^{c}
Crude fiber (%)	17.20 ± 0.14^{a}	10.90 ± 0.80^{c}	12.12 ± 0.20^{b}
Ash (%)	8.14 ± 0.14^{b}	8.36 ± 0.10^{b}	13.02 ± 0.40^{a}
Total Carbohydrates (%)	34.32 ± 0.88^{b}	41.25 ± 0.35^{a}	36.07 ± 1.00^{b}
Caloric value (kcal per 100 g)	341.20 ± 1.20^{b}	356.20 ± 2.06^{a}	300.66 ± 0.58^{c}
TPC (mgGAE/100g)	127.00 ± 0.03^{a}	79.10 ± 0.04^{b}	66.51 ± 0.90^{c}
TFC (mgQE/100g)	6.43 ± 0.15^{c}	8.70 ± 0.20^{b}	13.33 ± 0.38^{a}
DPPH (%)	89.91 ± 0.72^{a}	41.48 ± 1.01^{b}	34.86 ± 0.92^{c}
ABTS (%)	91.35 ± 0.50^{a}	63.56 ± 0.80^{c}	66.71 ± 0.15^{b}

Results are expressed as mean ± standard deviation. Different letters in the same line are significantly different. TPC, total phenolic compounds; GAE, gallic acid equivalent; TFC, total flavonoid compounds; QE, quercetin equivalent; DPPH, free radical scavenging DPPH; ABTS, free radical scavenging ABTS.

Table 2. Decrease and increase in total phenolic compounds (TPC) and total flavonoid compounds (TFC) between wort before fermentation (WBF) and beers with (a) Rubim and (b) Mastruz

Treatments				
(a)	TPC	*p*-value	TFC	*p*-value
BS	-21.00%	0.0001	+14.00%	0.001
BR25	-19.50%	0.0001	-15.00%	0.001
BR50	-12.00%	0.0001	+ 5.60%	0.010
BR75	-25.00%	0.0001	+ 5.00%	0.084
BR100	-23.00%	0.0001	+ 3.00%	0.117
(b)	TPC	*p*-value	TFC	*p*-value
BS	-21.00%	0.0001	+14.00%	0.001
BM25	-15.00%	0.0001	-14.00%	0.0001
BM50	-20.00%	0.0001	+13.50%	0.0001
BM75	-16.00%	0.0001	+ 4.70%	0.025
BM100	+ 8.50%	0.0001	+16.00%	0.001

Significance level at $P \leq 0.05$. BS, beer standard; BR25, beer with hop replacement by 25% Rubim; BR50, beer with hop replacement by 50% Rubim; BR75, beer with hop replacement by 75% Rubim; BR100, beer with hop replacement by 100% Rubim; BM25, beer with hop replacement by 25% Mastruz; BM50, beer with hop replacement by 50% Mastruz; BM75, beer with hop replacement by 75% Mastruz; BM100, beer with hop replacement by 100% Mastruz.

Table 3. Decrease and increase in DPPH and ABTS assay between wort before fermentation (WBF) and beers with (a) Rubim and (b) Mastruz

Treatments				
(a)	DPPH	*p*-value	ABTS	*p*-value
BS	- 4.50%	0.028	+25.0%	0.001
BR25	-21.0%	0.0001	-13.0%	0.007
BR50	-15.0%	0.001	+11.0%	0.044
BR75	-33.0%	0.0001	-33.0%	0.0001
BR100	-11.0%	0.006	-38.0%	0.0001
(b)	DPPH	*p*-value	ABTS	*p*-value
BS	- 4.50%	0.028	+25.0%	0.001
BM25	-39.0%	0.0001	-33.0%	0.0001
BM50	-49.0%	0.0001	-42.0%	0.0001
BM75	-43.0%	0.0001	-34.0%	0.0001
BM100	-18.0%	0.0001	-25.0%	0.0001

Significance level at $P \leq 0.05$. BS, beer standard; BR25, beer with hop replacement by 25% Rubim; BR50, beer with hop replacement by 50% Rubim; BR75, beer with hop replacement by 75% Rubim; BR100, beer with hop replacement by 100% Rubim; BM25, beer with hop replacement by 25% Mastruz; BM50, beer with hop replacement by 50% Mastruz; BM75, beer with hop replacement by 75% Mastruz; BM100, beer with hop replacement by 100% Mastruz.

Table 4. Pearson's Correlation Coefficient (R) between different antioxidant capacity (DPPH and ABTS assay) parameters, total phenolic compounds (TPC), total flavonoid compounds (TFC) and International Bitterness Units (IBU) in beers with (a) Rubim and (b) Mastruz

Parameters			
(a)	DPPH	ABTS	IBU
TPC	-0.063[a]	0.971**	0.335[a]
TFC	-0.155[a]	0.723*	0.121[a]
IBU	-0.444[a]	0.267[a]	
(b)	DPPH	ABTS	IBU
TPC	0.955**	0.951**	-0.305[a]
TFC	0.667*	0.596[a]	-0.760*
IBU	-0.163[a]	-0.053[a]	

** Significance level at $P \leq 0.01$.
[a]Not significant at $P \leq 0.05$ level.
*Significance level at $P \leq 0.05$.

ARTICLE 2

Influence of gender in acceptability of beers made with medicinal plants

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Abstract

An increase in consumption and interest in craft and specialized beer by consumers, makes interesting for breweries incorporating new flavors and ingredients on beer. The aim of this study was to determine physical-chemical properties, acceptability by consumers and gender behavior by beer with Rubim (*Leonurus sibiricus*) and Mastruz (*Chenopodium ambrosioides* L.) as hop bitterness substitution, following the proportion: 25%, 50%, 75% and 100%. A consumer trial was conducted to determine overall acceptance of nine different beers. The participants were asked to rate their liking of color, aroma, flavor, and bitterness intensity. Additionally, participants assessed purchase intention. Hop bitterness substitution affected physical-chemical and sensory properties of beers. IBU decreased with increased of hop bitterness substitution and bitterness sensorial was felt by men and women in beers. All beer presented good purchase intention for men and women, less BR100 which receipted lowest overall liking and purchase intention. BR100 presented lowest pH, higher acidity. Gender influenced in choices of beers styles and results showed that medicinal plants can be substitute for hop.

Keywords: Plant beer, consumer acceptance, gender preferences.

1. Introduction

Beer is one of oldest drinks and most consumed alcoholic beverage in the world (Kawa-Rygielska, et al., 2019). It is produced by brewing process by *Saccharomyces cerevisiae* that turn fermentable sugars from malt wort mostly in ethanol and flavor-determining sub-products (Denby et al., 2018; Rygielska, et al., 2019). The main raw material used for brewing process is malt and hops (*Humulus lupulus*), that ensuring rich beverage in carbohydrates, amino acids, vitamins, and phenolic compounds (Sohrabvandi, et al., 2012). Majority phenolic compounds present in beer are from malt (70-80%) and a smaller part from hop (Callemien and Collin, 2009; Ducruet et al., 2017).

A growing segment in beverage industry is craft beer, having as a main characteristic, flavor, and manufacturing techniques (Oliver, 2011). Global market of beer is dominated by traditional beers and produced large-scale beers. Craft beers segment competes with beers quality and diversity (Marongiu et al., 2015; Ducruet, et al., 2017). Frequent beer consumers are seeking for beers with improved characteristics than offered the commercial brands (Aquilani et al., 2015). Craft beers manufacturing with traditional methods, differ from each other by addition of non-conventional ingredients. Hops may be out or agreed with spices, herbs, or vegetables, considering the beer style (Kleban and Nickerson, 2012; Martínez et al., 2017).

Hops were used as preservative agent for centuries, their aroma pleased consumers and become one of principal ingredient (Schönberger and Kostelecky, 2011). Different types of special beers were product in world, differing on composition of raw and flavorings materials (Hornsey, 2003; Dordevic et al., 2016). Medicinal plants are commonly known by bioactive compounds, as terpenes and phenolic compounds (flavonoids and phenolic acids), which are effective as food additives (Cai et al., 2004; Ortega-Ramirez et al., 2014). Flavonoids are usually responsible for color, taste, lipid oxidation prevention, vitamins, and enzymes protection (Yao et al., 2004; Kumar and Pandey, 2013).

Rubim (*Leonurus sibiricus*) is a medicinal plant native of Siberia, China, Korea, Japan, and Vietnam (Zachow et al., 2017). Used as cooking ingredient and therapeutic form, is effective in treatment of diabetes, viral infections, headache, and respiratory diseases (Lorenzi and Matos, 2002; Zachow et al., 2017). The potential analgesic, anti-inflammatory, antioxidant and anti-bacterial are directly linked with bioactive compounds present in their composition, such as diterpenes, triterpenes, flavonoids, and phenolic compounds (Sayed et al., 2016). Mastruz (*Chenopodium ambrosioides* L.) is an herbaceous plant, native of Central and South American

(Barros et al., 2013). Has potential anti-inflammatory, anticarcinogenic, antimicrobial, among others (Podolak et al., 2016; Zohra et al., 2018). Flavonoids and terpenoids are found in large amounts in their composition (Zohra et., 2018).

Bioactive compounds, such as phenolic compounds are associated with bitterness in plants and teas (Ares et al., 2009; Ma et al., 2014). Astringent and bitter foods are more prone to reject by consumers (Bate-Smith, 1954; Drewnowski and Gomez-Carneros, 2000). Usually, bitterness is the principal attribute of beer (Hayward et al., 2019), liking this attribute, depends on many factors, such as gender and age (Drewnowski, 2001). Breweries looking for new flavors, introducing new ingredients for manufacturing. Rubim and Mastruz might be a potentially source of new flavors, as substitute of bitterness from hops, impart less bitterness, working more as an aromatic hop than bitterness hop, making beverages more acceptable to wide range of consumers.

No studies with Rubim and Mastruz as substitution of hop in alcoholic beverage and acceptance of consumers. Studies of new ingredients, understanding the added value into healthy characters, and their importance in acceptance of consumers, will help brewers develop new types of beers, accepted for a wider public. Partial or total hop bitterness substitution with addition of new ingredients may satisfy consumers' palates and attribute new flavors and characteristics for beers. The aim of this study is investigated physical-chemical properties of beers produce with partial or total hop bitterness substitution by Mastruz and Rubim, linking with beers characteristics, acceptability by consumers and gender behavior.

2. Material and methods

2.1 Beer manufacturing

Beers were produced by a modified ale-type beer brewing method, with addition of select medicinal plants (Rubim and Mastruz). Wort was prepared, using commercially Pilsen malt according to following mashing programmer: 30 min at 44ºC, 20 min at 52ºC, and 30 min at 70ºC. The mash was then heated to 76ºC and filtered to yield wort, which was kept boiling for 60 min at 98ºC. Bittering agents (hops, Rubim or Mastruz) were added at beginning of boiling. The hop bitterness substitution was based on global bitterness (IBU) of hop, Rubim and Mastruz: 40, 12 and 25, respectively. Medicinal plants proportion were 25%, 50%, 75%, and 100% from hop bitterness. Plants has not mixed each other. Nine beer formulations were produced, with the beer standard. After cooling, US-05 yeast was added, and fermentation was carried out at 20ºC for 7 days. Maturation was held at 3ºC for two weeks. Beers were stored at amber glass bottles, sugar (3g/L) was added to promote carbonation, at 23ºC for a week. Beers have been pasteurized (65ºC at 10 min).

2.2 Physical-chemical parameters of beer

Physical-chemical parameters of beer analyzed were pH (Tecnopon, mPA-210), total soluble solids (Refractometer HI 96801. Nusfalau, Romania), acidity, alcohol by volume (ABV), bitterness (IBU), EBC (Color) (Chroma Meter CR-400. Minolta, USA), and were conducted using official methods of Analytical Division of European Brewery Convention (European Brewery Convention, 1987). All measurements were performed in triplicate.

2.3 Consumer testing

Analyzes was approved by the Research Ethics Committee at Universidade Estadual de Maringá (CAAE: 39847220.8.0000.0104). Participants were recruited by online classified advertisements. Eighty-four regular beer consumers were recruited, included students and professors at the University. Consumer's participants, before analyzes, were asked to complete a questionnaire consisting of questions about their demographics, interest in beer, knowledge about beer, and beer consumption habits, and the focus group. Participants were divided into groups according to their gender and consumption frequency: Frequent Consumers (participants whose answers were "once a week" and "twice a week") and Not Frequent Consumers (participants whose answers "once a month" and "twice a month")

Beers were served in acrylic glasses coded with random three-digit numbers. 30 mL of the beer samples was served, at 4ºC. Water was provided for mouth rinsing between the beer samples tastings. Consumers were asked to look and drink each beer and to answer questions regarding their linking of the color, aroma, flavor, overall acceptance on a structured 9-point hedonic scale anchored with 'dislike extremely' and 'like extremely' (Meilgaard et al., 1999; García-Gómez et al., 2019). A medium score was excluded, according to Font i Furnols et al. (2008). Bitterness intensity was evaluated using a structured five-point scale (1 = extremely intense to 5 = little intense) (Reis and Minim, 2010). Purchase intention of beers was assessed at the same time; results were expressed in percentage.

2.4 Statistical analyzes

Hedonic values obtained from color, aroma, flavor, overall acceptance, and bitterness intensity, were analyses using a 2-way ANOVA. Post hoc Tukey's test was completed to determine if they were any significant differences in linking among the different treatments. Differences were considered significant at $P < 0.05$. Simple Correspondence Analyzes was performed graphically to show the preferences of identified groups together with the gender variable towards beer types mentioned by participants.

Principal Component Analyzes (PCA) was performed to find relationships between different parameters (treatments, sensory testing, and beer physical-chemical parameters) and to detect possible clusters within variables. A hierarchical clustering (with Ward's method and Euclidean distance) was completed, using results from overall acceptance. All analyzes was conducted using SPSS (v.20.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) and Statistica 10.0 software (Start Soft Inc., USA).

3. Results and discussion

3.1 Characteristics of beers

Results of beer physical-chemical parameters with Rubim and Mastruz are shown in Table 1. The hop bitterness substitution by Rubim and Mastruz to brewing did not have a significant effect in beer total soluble solids, and ABV.

BR100 showed significant difference in pH and acidity values. Lowest pH and consequently higher acidity. Beer colloidal instability depend on low pH (~4.4), and sensorially, lowest pH intensified astringency (Siebert and Chassy, 2004; François et al., 2006). BR50 presented significant difference in EBC, during pasteurization can occur a thermal absorption in beer, which explains degradation of polyphenols, formation of Maillard products and color increment (Cao et al., 2011). IBU of beers decreased with increased of hop bitterness substitution. The boiling process produces compounds on the main source of bitterness in beers – iso-α-acids (isohumulones), from hop α-acids (humulones) (Oladokun et al., 2017). Bitter acids (IBU) are an analytical measure of the expected quantity in beer and gives an approximate value of iso-α-acids in milligram per litre of beer (Hough et al., 1982). Rubim and Mastruz presents in its chemical composition phenolic compounds and low IBU (Barros et al., 2013; Sayed et al., 2016; Oliveira et al., 2017; Zohra et al., 2018). Lowest IBU values on beers with higher hop bitterness substitution can be linking with hot trub formation during boiling process, phenolic compounds and phenolic acids form complexes with proteins and are absorbed to hot trub or later to yeast cells during fermentation (Wannenmacher et al., 2018).

3.2 Consumption habits

Participants were requested about their beer preferences and were separated into groups ("Frequent Consumers" and "Not Frequent Consumers") (Table 2). The proportion of men and women regarding frequency of consumption is similar, which turns study by gender interesting. Correspondence Analyzes, the groups' preference towards the varieties of beer (Traditional beer vs. Craft Beer) with gender variable was observed (Figure 1), that women groups indicated one the most important attributes affecting acceptance of beverage products was low bitterness intensity (Muggah and McSweeney, 2017). These allow for a better understanding of consumer preferences towards this traditional drink. Men Not Frequent Consumers and Women Frequent Consumers group were positioned in the same quadrant as Craft Beer with Low Bitterness. Women Not Frequent Consumers was in the same quadrant as Traditional Beer with Low Bitterness. Men Consumers Frequent group was positioned at the same four-square as Craft Beer with High Bitterness, and Traditional Beer with High Bitterness.

Study on influence of gender about consumers' preferences has been researched. Based on gender, different tendencies were observed in results. Majority of men consumers buy beers in supermarkets (56.10%), barroom (34.14%), and convenience stores (9.76%). Women buy beers in supermarkets (59.52%), barroom (28.57%), and convenience stores (11.91%). Men place preference on flavor (51.17%) buying a beer, that is more important than price (25.58%), following by brand (23.25%). Sensory characteristics (flavor) is approximately twice as important as price and brand for men. Women place significance more than men on flavor (57.15%), price (23.81%) is approximately less trice important as flavor, and more important than brand (19.04%) for women. Research showed pleasure and distinct flavor of craft beers are important factors for consumers (men and women), also curiosity of new product and search for new experiences (Ribeiro et al., 2020).

Men and women have differences in type of beer consumption (Figure 1), and when buy a new beer (Figure 2). Correspondence Analyzes was performed to find relation between gender and factors considered by consumers to taste a new beverage style. Psychological factors can influence consumers' decisions. Men from this study was influenced by friend's recommendation when choosing a new beverage style. Studies stated previous sensorial experiences affect present consumers' choice (Sester et al., 2013; Aquilani et al., 2015). Women was influenced mostly by "tasting new styles" when choosing a new beverage. Other study reveals that flavor is more important than prince and brand when consumers selecting a beer to buy, mentioning labelling/packaging, recommendation from others, and brewery specifications (Gabrielyan et al., 2014).

Gender differences in alcohol consumption are considered as one the few universal difference in human social behaviour (Holmila and Raitasalo, 2005). Men tended consume beer to socialize, while women perceived more functional benefits (Ma and Mustonen 2000; Holmila and Raitasalo, 2004). Distinct studies showed that men outnumber women as consumers of speciality or craft beers (Brasseurs du Nord., 2013; Aquilani et al., 2015). Men consumers drink beer to reinforce masculinity and group inclusion (Gómez-Corona et al., 2017). Women become more and more attracted to beer as consequence of publicity specifically directed at them, healthy characters, and versatility of this beverage (Donadini et al., 2013). This association between frequency and gender should be done more research. While craft beers are described as having new or rich, and flavor characteristics intense, is possibility some craft beer drinkers more be attracted by authenticity of beer, its local quality, or partnerships that exists when one is a member of drinking segment (Figure 2) than intense flavor characteristics (Donadini and Porretta, 2017; Jaeger et al., 2020). Gender research's shows women prefer complex flavors and less bitterness in beer, unlike the men (Guinard et al., 2000; Donadini et al., 2016). Craft beers have greater intensity of bitterness may be the reason women dislike. It was found bitterness is a key factor of dislike for women consumers in trial. Men where more frequently consumers of beer are directly tied to more variable beer choice and looked stronger flavors in beverage choices (Donadini et al., 2014).

3.3 Beers sensory by gender

Specific sensory analyzes based on gender is shown in Table 3. Men and women had bitterness perception even low or none IBU presented on beers (Table 3). Beers with Rubim and Mastruz as hop bitterness substitution showed less or none IBU (Table 1). Impart bitterness and aroma in beer is conventionally adds hops (*Humulus lupulus* L.) to wort and make boiling (De Keukeleire, 2000). Perception of bitterness is multifaceted. The bitterness facets, in addition to values acquired by analytical measurements provide a better overall impression of beer bitterness as perceived by consumers. Phytochemical characteristics of Rubim presents in its composition phenolic compounds, flavonoids, such as quercetin (Sayed et al., 2016; Oliveira et al., 2017). Flavonoids were the major phenolic compounds, being quercetin and kaempferol derivatives (Barros et al., 2013; Zohra et al., 2018) presents in chemical characterization of Mastruz. Polyphenol compounds are the main responsible for the bitterness and astringency of tea, red wine, and several types of fruits (Ares et al., 2009). Astringency is not a taste but a tactile sensation. Precipitation of salivary proteins diminished oral lubrication, which is usually described as dryness, puckering and rough-mouthfeel (Bate-Smith, 1954). Flavan-3-ol monomers as (+) catechin and (-)-epicatechin provide beer astringency (Aron and Shellhammer, 2010), catechin was found in studies about major polyphenols in Rubim. Hydroxybenzoic acids have been shown to elicit sensation of astringency (Lesschaeve and Noble, 2005), phenolic acid identified in Rubim composition (Sitarek et al., 2017). Mastruz and Rubim are rich in phenolic compounds, which contribute on beer bitterness, as flavan-3-ols and tannins are related to bitterness and astringency in beverages (Radonjić et al., 2020). Phenolics compounds of flavonoid family provide beer bitterness and astringency, perceived organoleptically (Robichaud et al., 1990; Kielhorm et al., 1999; Aron and Shellhammer, 2010).

Beer made with medicinal plants as hop bitterness substitutes were not significantly different from each other or beer standard for overall acceptance, and bitterness intensity for men. Significant differences were found for color between BS, BM50 and BR100, that showed lowest color acceptance for men. Men are less tasters than women, than seek for new flavors (Figure 2). BS was beer more accepted with greater scores for aroma and flavor, and BR100 was reject with lowest scores for aroma and flavor for women.

Principal component analyzes (PCA) is used to graphically present the relationship among the variables (physical-chemical properties and sensory attributes) for treatments. Experimental results are shown in Figure 3. PCA explained 77.97% of variables in two axles P1 (55.21%) and P2 (22.76%) for men (Figure 3a). PCA explained 80.52% of variables for women (Figure 3b). Attributes of aroma and overall acceptance for men are on right side of P1, located in same four-square to BS and BR25 (Figure 3a). BM25, BM50 and BM75 were also at right side of P1, different four-square and close to bitterness intensity, due to hop bitterness substitution for Mastruz and Rubim (Figure 3a). Men frequent beer consumers prefer Craft beer and Traditional beer with higher bitterness (Figure 1), corroborating with PCA (Figure 3a). Beers with hop bitterness substitution presented lowest IBU (Table 1), bitterness sensorial is equally for all beers. Attributes of aroma, flavor, and IBU for women are placed on right side of P1, located in same quadrant of BS, BR25 (Figure 3b). BM25 and BM75 contains higher concentrations of Mastruz, place at right side of P1, closed to bitterness intensity (Figure 3b). Women are considered more likely to be supertasters than men (Bartoshuk et al., 1994; McAnally et al., 2007), chose new styles when buy a beer (Figure 2), corroborating with BM100 are in the same quadrant of BS. It should be considered the fact many of tastes and food associations are acquired as result of experience (Spence et al., 2019). Consumers who prefer beers with stronger aroma, and greater bitter has association with frequency of consumer (Figure 1). Routine drinkers may have associate to less sensitivity bitterness and increased preference for alcoholic beverages (Andreeva et al., 2013; Jaeger et al., 2020). BR50 and BR100 were placed on the other side, inversely related to attributes for men and women (Figure 3).

Agglomerative hierarchical clusters (AHC) were used with view to recognize differences, similarities, preferences among consumers from results of consumers' sensory analyzes (overall acceptance). The number of groupings, or different profiles, is arbitrarily defined based on numeric measures and graphic representation in the dendrogram cluster. Analyzes carried out by Ward's method based on Euclidean distances. Three clusters were formed for both: men and women (Figure 4).

BS and BM25 are close as can see in PCA (Figure 3). BR50, BR75 and BR100 are the most distant group for men and have lowest IBU (Table 1). The most distant agglomerate was between BR50 and BR100, these beers presented lowest overall acceptance for women (Table 3). Results for women presented agglomerate between BR25 and BM100 (Figure 4b), showing the new style of beer, considered a craft beer satisfy and please women beverage drinkers. Consumers tend to assimilate craft beers with the low flavor of traditional beer to their first exposure to craft beers, increased exposure to craft beers, consumers develop a preference for higher flavor profile (Figure 1). New styles of beer and curiosity towards craft beers (Aquilani et al., 2015; Hayward et al., 2019) are key factors for beers with bitterness hop substitution acceptance.

Purchase intention of beers are presented in Figure 5. In this study, intrinsic characteristics (color, aroma, bitterness) influence more than extrinsic characteristics (brand, price, alcohol content) in consumers purchase intention (sec. 3.2). Men would certainly buy BR25 (Figure 5a), with 49% purchase intention, corroborating with PCA (Figure 3a). The BR25, BR75, BM50 and BM75 are at same group (Figure 4a) for overall acceptance, consequently obtained great scores for purchase intention (Figure 5a). BS and BR25 had not different significant for aroma (Table 3), bitterness of BR25 was sensorially perceived for men and associated with BS bitterness, corroborating with correspondence analyzes (Figure 1), men frequent consumers prefer Traditional and Craft beer with higher bitterness, previously experiences affect present choice for men (Figure 2). Combination between 25% hop bitterness substitution by Rubim attracted men by authenticity of beer, these findings are supported by study with Danish consumers, men preferred strong beer, realized as local and identity product (Cardello et al., 2016). Beer standard obtained 52% of purchase intention for women (Figure 5b), highest score. The new flavors and aroma of BM50 and BM75 influenced in purchase intention and pleased women (Figure 5b). BR100 was reject for both: men and women, especially by women, 67%

(Figure 5b), that has higher acidity, lowest pH, and none IBU (Table 1), phenolic acids present on Rubim composition in higher quantity caused astringency, quality for dislike in beverages. Alcohol content, pH and total soluble solids can influence the astringency perception (Lesschaeve and Noble, 2005). Person have habits acquired in past experiences (Figure 1), yet consume and purchase for another reasons than habits, like functional benefit or to experience positive sensations' (Wood and Neal, 2009; Gómez-Corona et al., 2016).

4. Conclusion

Hop bitterness substitution by Rubim and Mastruz improved physical-chemical characteristics and sensory attributes of beers. Results showed men and women are different in preferences about beer style and when buy a beer. Women seek novelties, new flavors in beer, and are more tasting than men, which are more traditional and influential by friends. The hop bitterness substitution decreased IBU or showed none IBU, yet bitterness intensity is perceived by men and women in beers. BR100 was less accept beer for both, presented higher acidity and none IBU. The substitution of hop bitterness until 75% of Rubim and 100% of Mastruz are recommended. Behavior of consumers evaluate beers, and their acceptance of new hop bitterness substitutes will help brewing industry in product of new beverages.

Declarations of interest: none

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Author contribution

Anderson Lazzari: Investigation, Writing - Original Draft, Writing - Review & Editing, Project administration, Formal analysis, Conceptualization; Heloisa Dias Barbosa: Investigation; Evandro Ribeiro Machado Filho: Investigation; Ana Paula Dada: Investigation; Bianka Rocha Saraiva: Investigation; Paula Toshimi Matumoto-Pintro: Supervision, Writing - Review & Editing, Project administration, Conceptualization, Resources.

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Figure Caption

Fig. 1. Correspondence analyzes on preferences towards beers of the identified groups. TB: traditional beer; CB: craft beer; HB: high bitterness; LB: low bitterness.

Fig. 2. Correspondence analyzes between gender and factors considered by taste a new beverage style.

Fig. 3. Principal components analyze of beer sensory characteristics, color and IBU, (a) men and (b) women. IBU: International Bitterness Units; EBC: European Brewing Convention. BS, beer standard; BR25, beer with hop bitterness substitution by 25% Rubim; BR50, beer with hop bitterness substitution by 50% Rubim; BR75, beer with hop bitterness substitution by 75% Rubim; BR100, beer with hop bitterness substitution by 100% Rubim. BM25, beer with hop bitterness substitution with 25% Mastruz; BM50, beer with hop bitterness substitution by 50% Mastruz; BM75, beer with hop bitterness substitution by 75% Mastruz; BM100, beer with hop bitterness substitution by 100% Mastruz.

Fig. 4. Dendrogram for overall acceptance of beer by gender effect (a) men and (b) women. BS, beer standard; BR25, beer with hop bitterness substitution by 25% Rubim; BR50, beer with hop bitterness substitution by 50% Rubim; BR75, beer with hop bitterness substitution by 75% Rubim; BR100, beer with hop bitterness substitution by 100% Rubim. BM25, beer with hop bitterness substitution with 25% Mastruz; BM50, beer with hop bitterness substitution by 50% Mastruz; BM75, beer with hop bitterness substitution by 75% Mastruz; BM100, beer with hop bitterness substitution by 100% Mastruz.

Fig. 5. Purchase intention of beers by gender (a) men and (b) women. BS, beer standard; BR25, beer with hop bitterness substitution by 25% Rubim; BR50, beer with hop bitterness substitution by 50% Rubim; BR75, beer with hop bitterness substitution by 75% Rubim; BR100, beer with hop bitterness substitution by 100% Rubim. BM25, beer with hop bitterness substitution with 25% Mastruz; BM50, beer with hop bitterness substitution by 50% Mastruz; BM75, beer with hop bitterness substitution by 75% Mastruz; BM100, beer with hop bitterness substitution by 100% Mastruz.

Fig. 1

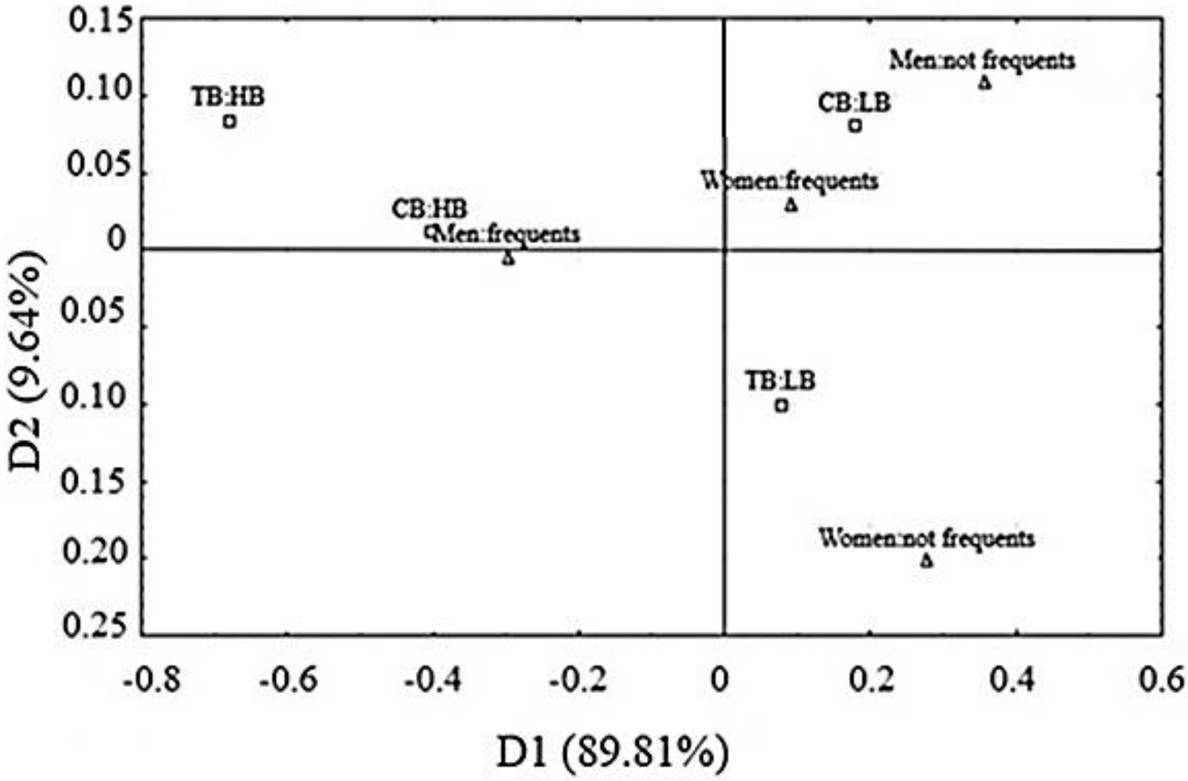

0.15
0.10
0.05
0
0.05
0.10
0.15
0.20
0.25
D2 (9.64%)
TB:HB
CB:HB
Men:frequents
Men:not frequents
CB:LB
Women:frequents
TB:LB
Women:not frequents
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
D1 (89.81%)

Fig. 2

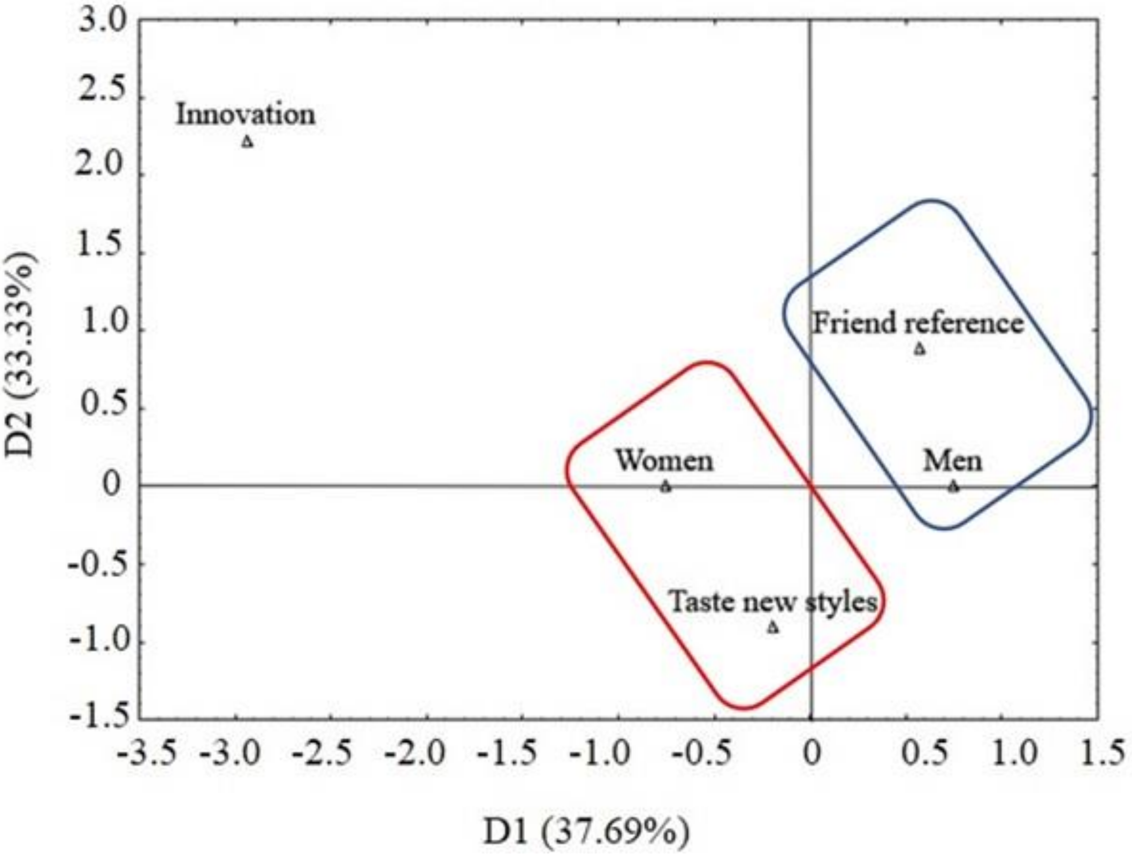
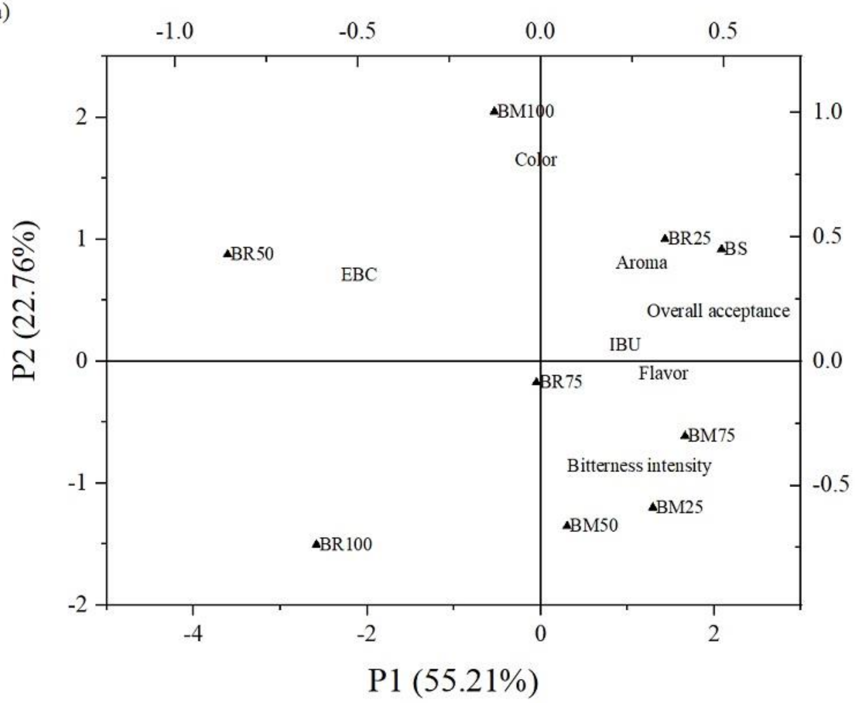

Innovation
Friend reference
Women
Men
Taste new styles
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
1.5
D2 (33.33%)
D1 (37.69%)

Fig. 3

(a)


BM100
Color
BR50
EBC
BR25
BS
Aroma
Overall acceptance
IBU
Flavor
BR75
BM75
Bitterness intensity
BM25
BM50
BR100
P1 (55.21%)
P2 (22.76%)

(b)

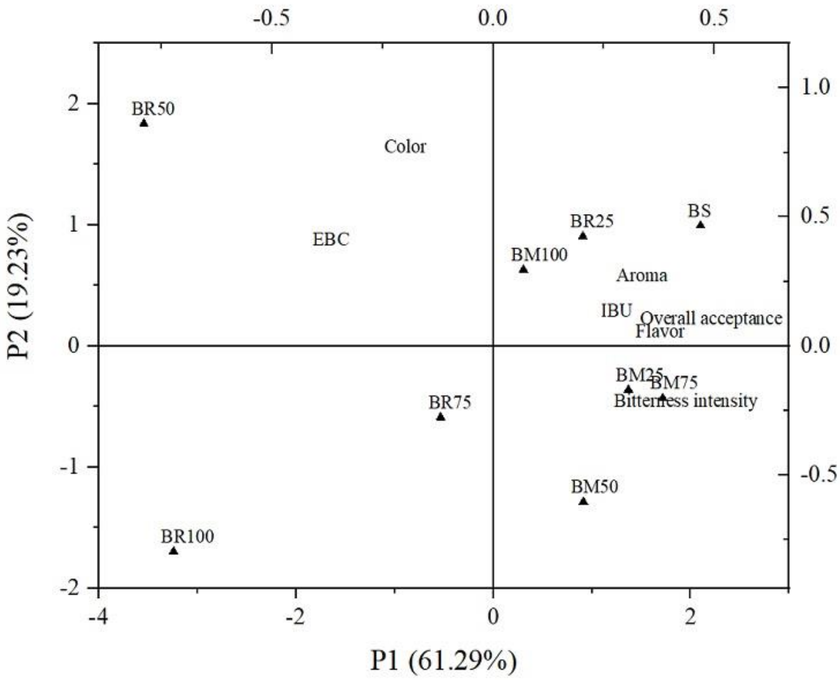

BR50
Color
EBC
BR25
BS
BM100
Aroma
IBU
Overall acceptance
Flavor
BM25
BM75
Bitterness intensity
BR75
BM50
BR100
P1 (61.29%)
P2 (19.23%)

Fig. 4

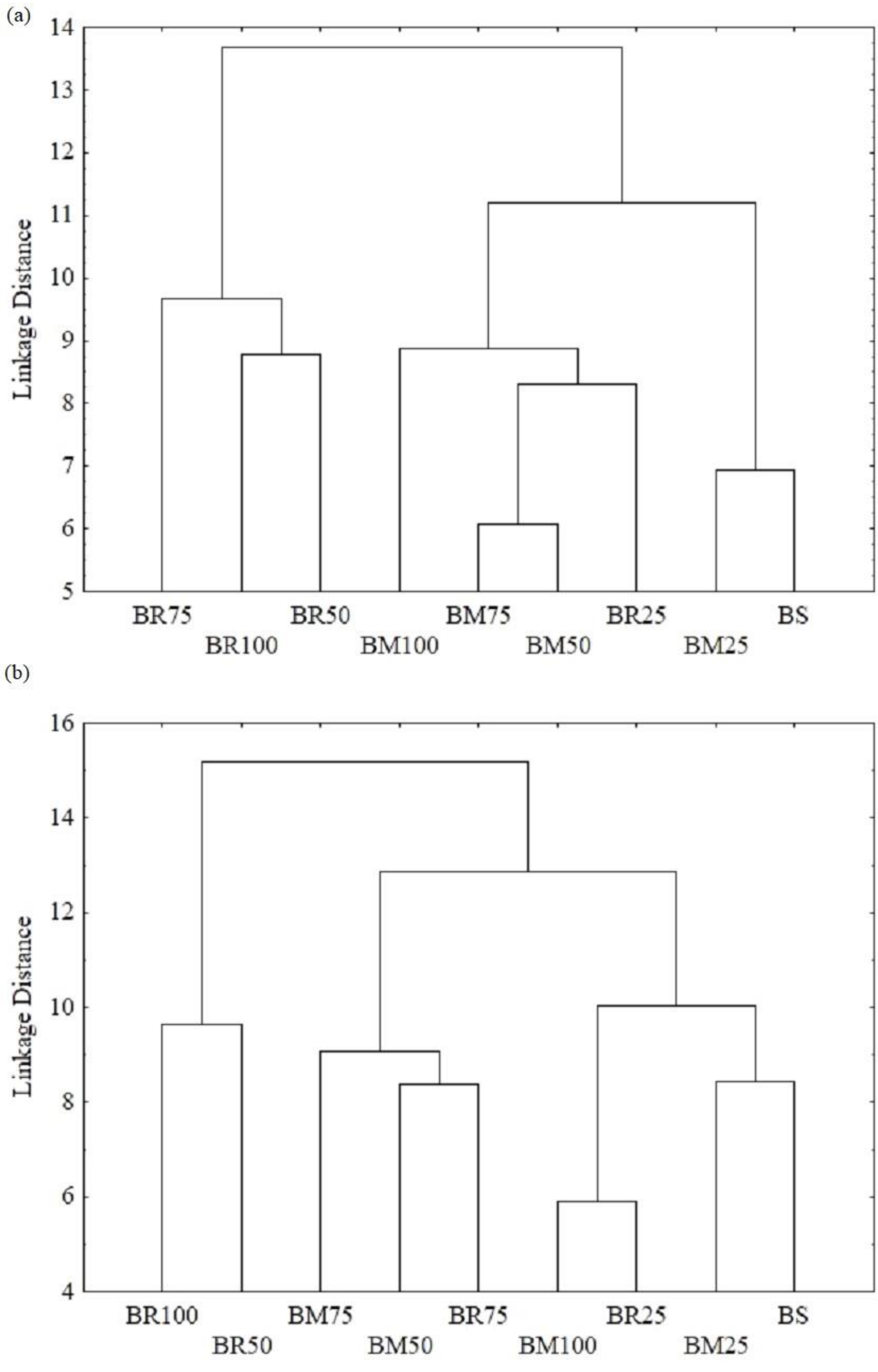
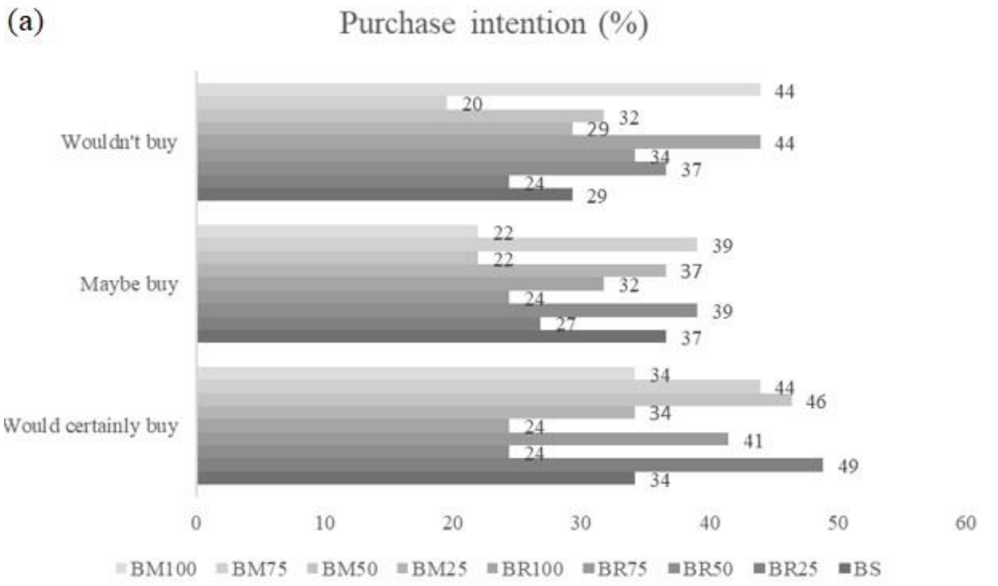

(a)
Linkage Distance
14
13
12
11
10
9
8
7
6
5
BR75
BR100
BR50
BM100
BM75
BM50
BR25
BM25
BS
(b)
Linkage Distance
16
14
12
10
8
6
4
BR100
BR50
BM75
BM50
BR75
BM100
BR25
BM25
BS

Fig. 5

(a)


Purchase intention (%)
Wouldn't buy
Maybe buy
Would certainly buy
44
20
32
29
44
34
37
24
29
22
39
22
37
32
24
39
27
37
34
44
46
34
24
41
24
49
34
0
10
20
30
40
50
60
BM100
BM75
BM50
BM25
BR100
BR75
BR50
BR25
BS

(b)

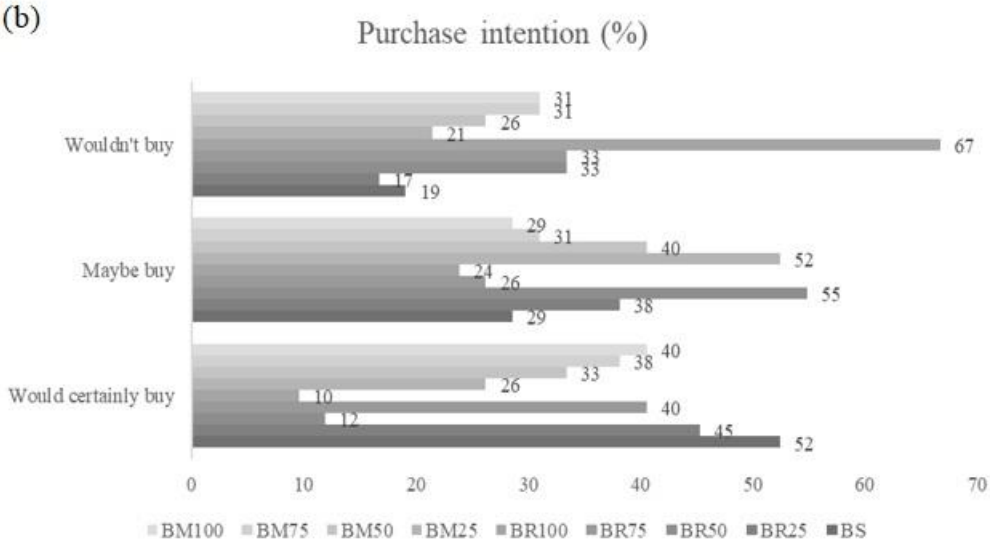

Purchase intention (%)
Wouldn't buy
Maybe buy
Would certainly buy
31
31
26
21
67
33
33
17
19
29
31
40
52
24
26
55
38
29
40
38
33
26
10
40
12
45
52
0
10
20
30
40
50
60
70
BM100
BM75
BM50
BM25
BR100
BR75
BR50
BR25
BS

Table 1. Physical-chemical properties of beer with Rubim or Mastruz

	pH	Acidity	ºBrix	ABV (%)	EBC (Color)	IBU
BS	5.17 ± 0.34^{ab}	0.13 ± 0.02^{bc}	5.93 ± 0.05	5.36 ± 0.25	4.71 ± 0.81^{b}	7.22 ± 0.03^{a}
BR25	5.26 ± 0.18^{a}	0.13 ± 0.01^{bc}	6.06 ± 0.20	5.86 ± 0.32	5.28 ± 0.51^{b}	3.95 ± 0.07^{b}
BR50	5.30 ± 0.03^{a}	0.11 ± 0.02^{c}	5.36 ± 0.32	5.13 ± 0.11	7.10 ± 0.96^{a}	1.25 ± 0.21^{e}
BR75	5.28 ± 0.07^{a}	0.12 ± 0.01^{bc}	5.73 ± 0.40	5.58 ± 0.30	5.43 ± 0.26^{b}	1.25 ± 0.21^{e}
BR100	4.90 ± 0.26^{b}	0.18 ± 0.03^{a}	5.90 ± 0.34	5.60 ± 0.55	4.86 ± 0.28^{b}	--
BM25	5.20 ± 0.08^{a}	0.13 ± 0.01^{bc}	5.73 ± 0.41	5.36 ± 0.55	5.24 ± 0.64^{b}	6.75 ± 0.21^{a}
BM50	5.20 ± 0.05^{a}	0.12 ± 0.01^{bc}	5.56 ± 0.55	5.16 ± 0.51	5.66 ± 1.13^{b}	4.20 ± 0.07^{b}
BM75	5.20 ± 0.04^{a}	0.13 ± 0.01^{bc}	6.06 ± 0.40	5.56 ± 0.20	4.73 ± 0.21^{b}	3.05 ± 0.07^{c}
BM100	5.04 ± 0.26^{ab}	0.15 ± 0.01^{ab}	5.83 ± 0.15	5.53 ± 0.46	5.78 ± 0.70^{b}	1.90 ± 0.14^{d}

Results are expressed as mean ± standard deviation. Different letters in the same column are significantly different ($P < 0.05$). ºBrix: total soluble solids; ABV: alcohol by volume; EBC: European Brewing Convention; IBU: International bitterness Units. BS, beer standard; BR25, beer with hop bitterness substitution by 25% Rubim; BR50, beer with hop bitterness substitution by 50% Rubim; BR75, beer with hop bitterness substitution by 75% Rubim; BR100, beer with hop bitterness substitution by 100% Rubim. BM25, beer with hop bitterness substitution with 25% Mastruz; BM50, beer with hop bitterness substitution by 50% Mastruz; BM75, beer with hop bitterness substitution by 75% Mastruz; BM100, beer with hop bitterness substitution by 100% Mastruz.

Table 2. Sociodemographic characteristics of identifying consumers' profile.

	Total (%)	Frequent (%)	Not Frequent (%)
Gender			
Men	51.20	76.74	23.26
Women	48.80	65.57	32.43
Age			
18-25	48.81	80.49	19.51
26-47	45.23	71.05	28.95
48-70	5.96	60.00	40.00
Education Level			
Low	2.40	100	0
Medium	33.33	71.42	28.53
High	64.28	77.77	22.23

Frequent: drink beer once and twice a week; Not frequent: drink beer once and twice a month.

Table 3. Specific beer consumption by gender (a) men and (b) women

(a)	Color	Aroma	Flavor	OA	BI
BS	7.63 ± 1.42^{a}	7.17 ± 1.14^{ab}	7.07 ± 1.23^{a}	7.17 ± 1.02	3.56 ± 0.95
BR25	7.43 ± 1.50^{ab}	7.20 ± 1.24^{ab}	7.27 ± 1.28^{a}	7.20 ± 1.10	3.49 ± 1.03
BR50	7.07 ± 1.38^{ab}	6.63 ± 1.40^{ab}	5.70 ± 1.72^{b}	6.30 ± 1.34	3.39 ± 1.16
BR75	7.03 ± 1.67^{ab}	7.10 ± 1.06^{ab}	6.60 ± 1.47^{ab}	6.90 ± 1.21	3.73 ± 0.97
BR100	6.33 ± 1.82^{b}	6.33 ± 1.40^{b}	6.47 ± 1.48^{ab}	6.33 ± 1.42	3.37 ± 1.06
BM25	6.53 ± 1.48^{ab}	7.00 ± 1.17^{ab}	7.20 ± 1.30^{a}	6.97 ± 1.24	3.71 ± 0.93
BM50	6.40 ± 1.61^{b}	7.03 ± 1.03^{ab}	6.90 ± 1.32^{a}	6.83 ± 1.20	3.78 ± 1.06
BM75	6.77 ± 1.33^{ab}	7.33 ± 1.21^{a}	7.07 ± 1.04^{a}	7.20 ± 0.84	3.73 ± 1.02
BM100	7.47 ± 1.25^{ab}	7.27 ± 1.23^{ab}	6.67 ± 1.50^{ab}	6.83 ± 1.17	3.20 ± 1.07
(b)	**Color**	**Aroma**	**Flavor**	**OA**	**BI**
BS	7.07 ± 1.64^{ab}	7.43 ± 1.16^{a}	7.07 ± 1.76^{a}	7.27 ± 1.08^{a}	3.60 ± 1.25
BR25	7.00 ± 1.62^{ab}	7.13 ± 1.38^{ab}	7.13 ± 1.38^{a}	7.23 ± 1.25^{ab}	3.50 ± 1.08
BR50	7.43 ± 1.13^{a}	6.40 ± 1.71^{ab}	6.07 ± 1.70^{ab}	6.33 ± 1.40^{ab}	3.26 ± 0.98
BR75	6.60 ± 1.87^{ab}	6.77 ± 1.52^{ab}	6.47 ± 1.63^{ab}	6.73 ± 1.46^{ab}	3.70 ± 1.02
BR100	6.30 ± 1.68^{ab}	6.17 ± 1.68^{b}	5.83 ± 1.53^{b}	6.23 ± 1.35^{b}	3.24 ± 1.22
BM25	6.37 ± 1.58^{ab}	6.93 ± 1.23^{ab}	7.10 ± 1.15^{a}	7.03 ± 1.21^{ab}	3.64 ± 1.10
BM50	5.87 ± 1.67^{b}	6.70 ± 1.68^{ab}	7.17 ± 1.55^{a}	7.07 ± 1.20^{ab}	3.67 ± 1.14
BM75	6.47 ± 1.90^{ab}	7.40 ± 1.45^{a}	7.03 ± 1.42^{ab}	7.23 ± 1.22^{ab}	3.70 ± 1.22
BM100	6.73 ± 1.72^{ab}	7.33 ± 1.10^{ab}	6.90 ± 1.30^{ab}	7.07 ± 0.94^{ab}	3.52 ± 1.30

OA: Overall acceptance; BI: Bitterness intensity. Results are expressed as mean ± standard deviation. Different letters in the same column are significantly different ($P < 0.05$). BS, beer standard; BR25, beer with hop bitterness substitution by 25% Rubim; BR50, beer with hop bitterness substitution by 50% Rubim; BR75, beer with hop bitterness substitution by 75% Rubim; BR100, beer with hop bitterness substitution by 100% Rubim. BM25, beer with hop bitterness substitution with 25% Mastruz; BM50, beer with hop bitterness substitution by 50% Mastruz; BM75, beer with hop bitterness substitution by 75% Mastruz; BM100, beer with hop bitterness substitution by 100% Mastruz.