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ORIGINAL ARTICLE

Production of low-alcoholic and low-gluten beer: physicochemical properties and volatile compounds

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Abstract

Gluten-free products and non-alcoholic beverages have become popular global trends. This study assessed strategies for producing low-alcoholic and low or gluten-free beers. Barley malts were fermented by *Saccharomyces cerevisiae* or *Saccharomycodes ludwigii*, and commercial endopeptidase was used to reduce gluten. *S. cerevisiae* is the most used yeast in alcoholic beer, so vacuum evaporation was used to reduce values of ethanol. *S. ludwigii* produces less alcohol, however, there was no data reported it would ferment on a semi-industrial scale. The physiochemical parameters in rice malt beers were similar to both yeasts. However, the parameters found differed for beer with barley malt mainly ethanol values, confirming the fermentative difference by tested strains. Volatile composition was evaluated and analyzed by multivariate analysis. The beers proposed by different methodologies: *S. cerevisiae* with barley malt, peptidase, and evaporation exhibited aromas of floral and featuring beer and spice notes, among others; *S. ludwigii* fermentation with barley malt and peptidase showcased aromas rich in floral and fresh accents, among others; and, *S. cerevisiae* with malted rice exhibited notes evoking fruity notes reminiscent of roses and *S. ludwigii* using malted rice was associated with aromas featuring fruity attributes and emitting rancid notes, among others. Among all the strategies tested, the one using barley malt, enzyme, and *S. cerevisiae* proved results in terms of aromatic parameters, even with the final boiling process to remove the alcohol.

Keywords: Aroma; New product; Low-alcoholic and gluten beer; Non-Saccharomyces; Biotechnology beverages.

Highlights

- *S. ludwigii* and *S. cerevisiae* in barley and rice malts with different conditions were tested
- Low gluten and low alcoholic beer with aroma quality were analyzed
- *S. ludwigii* was suitable to produce a low-alcoholic beverage

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1 Introduction

Beer is one of the most popular beverages worldwide, and the current development of new products is important to breweries to keep and/or enhance their market share. In this context, gluten-free and non-alcoholic and low alcohol beers are great trends. The demand for gluten-free products has grown in the past decade mainly due to allergic conditions and/or as part of a lifestyle and consequently, the global food market had been projected to grow from US$5.72 billion in 2021 to US$9.99 billion in 2028 (Fortune Business Insights, 2022). On the other hand, the non- and low-alcoholic beer (NAB) segment has observed huge improvement, mainly because new driving/drinking rules, health and religious reasons. This market is expected to amount to US$ 31.92 billion in 2022 and grow annually by 13.44% from 2022-2025 (Statistica, 2022).

For NAB production, several emerging technologies, mainly membrane technologies, have gained attention from the scientific community such as forward osmosis, reverse osmosis, and pervaporation (Jackowski & Trusek, 2018; Castro-Muñoz, 2019; Ambrosi et al., 2020), but due to financial aspects, they have not been applied in large scale. However, thermal treatment remains the most common dealcoholizing industrial process for beverages, mainly due to economic issues, and comprises evaporation under vacuum conditions and low temperatures (40-65 °C) to preserve the sensory properties by avoiding undesired secondary reactions, although it still causes the loss of the original aroma (Andrés-Iglesias et al., 2015; Mangindaan et al., 2018). As an alternative, biological strategies have been investigated. *Saccharomycodes ludwigii* has shown up to be an interesting NAB starter strain to produce a low alcoholic beer and high concentration of residual unfermented maltose (Bellut & Arendt, 2019). De Francesco et al. (2015) observed that *S. ludwigii* strains were more suitable for brewing low-alcohol beer, presenting high amounts of esters and a low of diacetyl, important aroma criteria for beverage acceptance. Among the most important factors influencing beer quality is the presence of well-adjusted amounts of higher alcohols and esters (Pires et al., 2014).

Prolamins are responsible for triggering the immune response of celiac disease allergic people and are named differently according to the cereal: gliadins in wheat and hordeins in barley. The industrial production of gluten-free beers (GFB) comprises mainly brewing alternative cereals or pseudo cereals materials or manufactured from gluten-containing cereals by reducing the level of gluten to below 20 ppm/L (Hager et al., 2014; Rubio-Flores & Serna-Saldivar, 2016). During the brewing process, some proteins are degraded during mashing, removed from wort during clarification, degraded by fermentation and/or decanted during maturation or beer clarification processes, reducing gluten content. However, non-malted rice has been used as a brewing GFB, since it is a cheap nutrient source, it consists of about 80% starch and its proteins are not considered coeliac toxic, although rice malt for beer production is rarely present in the literature (Rubio-Flores & Serna-Saldivar, 2016). Other important alternative to produce GFB is enzymatic treatment with peptidases, which break down gluten proteins into small peptides and would not be recognized by the immune system and would not cause reactions. Prolyl endopeptidase (PE) (EC 3.4.21.26), also known as postproline endopeptidase or prolyl oligopeptidase) is an enzyme that specifically hydrolyses peptide linkages occurring downstream of a proline residue and is the most commonly used in brewery, as it breaks down peptides in the carboxylic portion of proline residues, removing epitopes from gluten and helping to stabilize and prevent turbidity in the beer (Knorr et al., 2015; Bradauskiene et al., 2021). Also, it is stable to pH changes, and high alcohol content (Knorr et al., 2015), which are important features to utilization within brewery processes.

More detailed information about these different strategies to produce non/low-alcoholic and non/low-gluten beers is essential to proper insertion of the beverage on an industrial scale and the market. Thus, the objective of the present work was to evaluate beers brewed with both yeasts (*S. cerevisiae* and *S. ludwigii*) and added PE, as well as both yeasts in rice malt. Physicochemical and aromatic compounds were measured and deep discussion towards Brazilian legislation and the effect of treatments are presented.

2 Materials and methods

Experimental procedures took place at a microbrewery in Novo Hamburgo (RS, Brazil, latitude 29°41'5" S and longitude 51°8'31" W) and followed their industrial protocols.

2.1 Chemicals and ingredients

Pilsen, Munich, Carapils, Carahell malts, malted rice, wheat flakes, and hopes (cascade and citra) were purchased at a store specializing in ingredients for breweries (Novo Hamburgo, RS, Brazil). Calcium chloride, lactic acid and calcium sulphate were purchased from Êxodo Científica (Sumaré, SP, Brazil). Yeast nutrient was purchased from Hexis Científica (Jundiaí, SP, Brazil). Proline protease enzyme (PE) was purchased from White Labs (Clarity ferm, San Diego, California, USA); *S. cerevisiae* (Nottingham strain from Lallemand brand) was donated by a local brewery (Novo Hamburgo, RS, Brazil) and *S. ludwigii* were kindly donated by Levteck (Florianópolis, SC, Brazil). Other chemicals were obtained from Sigma-Aldrich (Saint Louis, Missouri, USA).

2.2 Beer production and treatments

Production protocols were based on previous tests and expertise of a local brewery. Four treatments were studied: *i)* barley malt and fermentation with *S. cerevisiae* and utilization of PE (BScP); *ii)* barley malt and fermentation with *S. ludwigii* and utilization of PE (BSlP); *iii)* rice and fermentation with *S. cerevisiae* (RSc); *iv)* rice and fermentation with *S. ludwigii* (RSl). All final beers' volume totalized 10 L. All treatments were conducted in duplicate.

Figure 1 shows the beers' production flowchart, which are detailed described below.

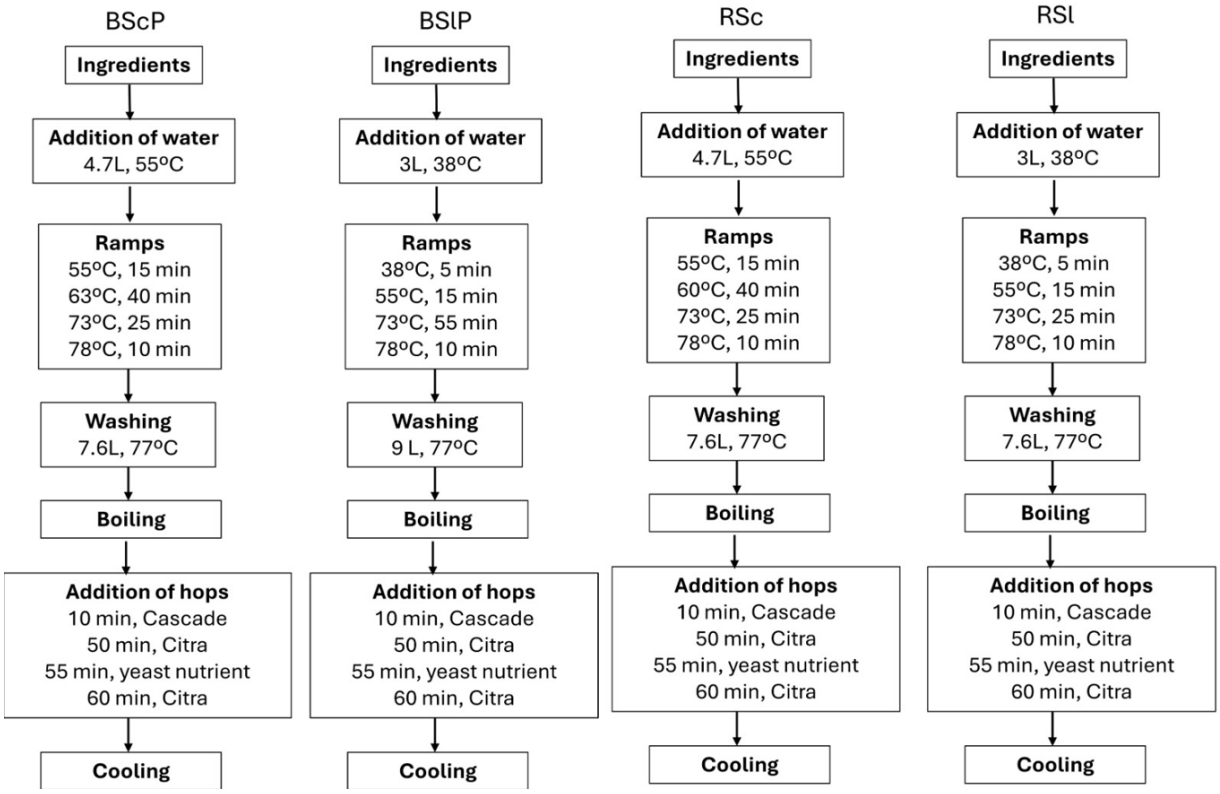

Figure 1. Flowchart of four treatments of beers production.

BScP was produced by mixing milled of 1,320 g of Pilsen malt, 120 g Munich malt and 105.3 g of wheat flakes to 4.7 L of water at 55 °C and the system was kept at 55 °C for 15 min, then 60 °C for 40 min, 73 °C for 25 min for the complete transformation of starch into minor and fermentable carbohydrates. Finally, the system was kept at 78 °C for 10 min to inactivate amylases. After the filtering process, 7.6 L of water at 77 °C was used to wash the spent grain. Wort was boiled and 27.1 g of Cascade hopes were added after 10 min and 2 g of Citra hopes after 50 min. After 55 minutes of boiling, 0.1 g of yeast nutrients were added. Finally, 25 g of cascade and 8.2 g of Citra hopes were added at 60 min. The system was cooled to room temperature and hopes removed. One hundred milliliters of S. *cerevisiae* were added and of 10 mL of PE. Fermentation happened at 16 °C for 7 days, cooled to 0 °C for 3 days when carbonic gas was added up to a pressure of 2 Bar in the barrel. To reduce alcohol content, BScP was treated with evaporation in rotavapor equipment (Q344B2 Quimis, Diadema, SP, Brazil) at 50 °C under vacuum (- 450 mmHg) for 45 min.

BSlP was produced by mixing milled 375 g of Pilsen malt, 300 g of Carapils malt, 200 g of Carahell malt and 60 g of wheat flakes to 3 L of water at 38 °C and the system was kept at 38 °C for 5 min, 55 °C for 15 min and 73 °C for 55 min for complete transformation of starch into minor carbohydrates. Finally, the system was kept at 78 °C for 10 min to inactivate amylases. After the filtering process, 9.0 L of water at 77 °C was used to wash the spent grain. Wort was boiled and 27.1 g of Cascade hopes were added after 10 min and 2 g of Citra hopes after 50 min. After 55 minutes of boiling, 0.1 g of yeast nutrients were added. Finally, 25 g of cascade and 8.2 g of Citra hopes were added at 60 min. The system was cooled to room temperature and hopes removed. One hundred milliliters of *S. ludwigii* were added and of 10 mL of PE. Fermentation happened at 16 °C for 7 days, cooled to 0 °C for 3 days when carbonic gas was added up to a pressure of 2 Bar in the barrel. No thermal treatment was done in these samples.

RSc was produced by mixing milled 1,550 g of malted rice with 4.7 L of water at 55 °C (which pH was previously corrected to ~5.5 with lactic acid) and the system was kept at 55 °C for 15 min, then 60 °C for 40 min, 73 °C for 25 min for complete transformation of starch into minor carbohydrates. Finally, the system as kept at 78 °C for 10 min to inactivate amylases. After the filtering process, 7.6 L of water at 77 °C was used to wash the spent grain. Wort was boiled and 27.1 g of Cascade hopes were added after 10 min and 2 g of Citra hopes after 50 min. After 55 minutes of boiling, 0.1 g of yeast nutrients were added. Finally, 25 g of cascade and 8.2 g of Citra hopes were added at 60 min. The system was cooled to room temperature and hopes removed. One hundred milliliters of *S. cerevisiae* were added. Fermentation happened at 16 °C for 7 days, cooled to 0 °C for 3 days when carbonic gas was added up to the pressure of 2 Bar in the barrel. To reduce alcohol content, RSc was treated with evaporation in rotavapor equipment (Q344B2 Quimis, Diadema, SP, Brazil) at 50 °C under vacuum (-450 mmHg) for 45 min.

RSl was produced by mixing milled 960 g of malted rice with 3 L of water at 38 °C (which pH was previously corrected to ~5.5 with lactic acid) and the system was kept at 38 °C for 5 min, 55 °C for 15 min and 73 °C for 55 min for complete transformation of starch into minor carbohydrates. Finally, the system was kept at 78 °C for 10 min to inactivate amylases. After the filtering process, 9 L of water at 77 °C was used to wash the spent grain. Wort was boiled and 27.1 g of Cascade hopes were added after 10 min and 2 g of Citra hopes after 50 min. After 55 minutes of boiling, 0.1 g of yeast nutrients were added. Finally, 25 g of cascade and 8.2 g of Citra hopes were added at 60 min. The system was cooled to room temperature and hopes removed. One hundred milliliters of *S. ludwigii* were added and fermentation happened at 16 °C for 7 days, cooled to 0 °C for 3 days when carbonic gas was added up to the pressure of 2 Bar in the barrel. No thermal treatment was done in these samples.

2.3 Physicochemical analyses

2.3.1 Analysis of original extract, apparent extract and alcohol

The analysis of these parameters was performed with the equipment that measures the alcohol content of beer by the absorbance in bands close to the infrared (NIR-Near-infrared). Density is determined using a digital densimeter attached to the equipment. With these values, the Alex equipment (Anton-PAAR, Graz,

Austria) provides results of alcohol content, apparent extract, real extract, original extract, calories, among others (Table 1). For this, samples were prepared according to the manufacturer's instructions, degassed in an ultrasonic bath, filtered through filter paper with the aid of diatomaceous earth and inserted into the equipment utilizing a peristaltic pump (ASBC Beer - 4 Alcohol, 2004). Original Gravity (OG) of the wort and Final Gravity (FG) of the beer were measured by their density and expressed as °P. The alcohol content was expressed as % v/v.

2.3.2 Color

Color analyzes were performed by absorbance at 430 nm at UV/Vis spectrophotometer (UV-1600, Pró-Análise, Brazil), using mineral water as blank. Results were multiplied by 1.27 to express results as SRM and by 1.97 to express results as EBC (ASBC Beer - 10 Color, 2015).

2.3.3 Immunoassay for quantification of gluten

The immunoassay for gluten detection was performed by the competitive R5 Elisa method, which is capable of detecting hydrolyzed prolamins in beer. The kit for analysis is the Ridascreen® Gliadin competitive (R-Biopharm, Darmstadt, Germany) where the monoclonal antibody R5 recognizes peptide sequences of wheat gliadins and malt prolamins with a detection limit of 10 ppm gluten. The calibration curve was performed with the patterns that are composed of a hydrolyzed mixture of wheat, malt and rye. Sample preparation was performed by adding 1 mL of beer to 9 mL of 60% (v/v) ethanol and 10% fish gelatin (m/v) solution. The sample was then mixed in a vortex, centrifuged for 10 min at 2,500 ×g and the supernatant was diluted 1:50 with the kit diluent. The measurement was performed in a microplate reader at 450 nm and the absorption is inversely proportional to the gliadin concentration.

2.4 Volatile compounds

The volatile compounds were extracted using the headspace solid-phase microextraction technique (HS-SPME). DVB/Car/PDMS fibers (Supelco®, Sigma Aldrich, Saint Louis, Missouri, USA; 50/30 µm, 10 mm long) were used in the procedure. Fifty milliliters of the sample were degassed in an ultrasound bath (Unique®, USC-800, Indaiatuba, SP, Brazil) at 5 °C for 20 min. Sample aliquots of 5 mL and 1.5 g of sodium chloride (Merck®, Darmstadt, Germany) were added in 20 mL vials and immediately sealed with a screw cap containing a polytetrafluoroethylene (PTFE) septum. Volatile compounds extraction was carried out at 35 °C with 50 min of fiber exposure to the sample headspace. The vial containing the sample was maintained for 5 min under the same conditions prior to the extraction. The sample was stirred throughout the entire analysis period.

Determination of volatile compounds was performed using a gas chromatograph coupled to a mass spectrometer (GC/MS), (Shimadzu Corporation QP2010-Plus, Kyoto, JP). The SPME fiber containing the compounds was thermally desorbed into the GC/MS injector at 250 °C for 10 min, operating in the splitless mode (splitter valve closed for 1 min) off containing the isolate. Analytes were separated using a SupelcoWax-10 column (30 m × 0.25 mm; 0.25 µm of thickness film; Supelco®, Sigma-Aldrich, Saint Louis, Missouri, USA). The temperature program of the column started at 35 °C for 5 min, followed by an increasing ramp of 5 °C min^{-1} until reaching 230 °C where it remained in isothermal conditions for 5 min. Helium (purity grade 5.0; White Martins, Osasco, SP, Brazil) was used as a carrier gas with a constant linear velocity of 39.4 cm s^{-1}. The GC/MS interface and the ionization source were maintained at 230 and 250 °C, respectively. The quadrupole mass analyzer was operated in the scan mode (35-350 *m*/*z*). Volatile compounds quantification was performed through internal standardization by adding the internal standard 3-octanol (10 µL of ethanol solution at 86 g/L) to the sample according to Bertagnolli et al. (2017). Volatile compounds were identified by comparing the experimental mass spectra with those found in the spectral library of the

National Institute of Standards and Technology (NIST 05s) or positively identified when compared with those standards mass spectrum of available esters, ketones, acids, alcohols, aldehydes, and terpenes, as indicated in Table 2. In addition, the experimental linear retention indexes (LRI) were calculated using alkane series retention times (C7-C30) and used to match the experimental LRI with those available in the literature. Identified compounds were related to their representative odor descriptor using the recognized flavor site flavornet.org (www.flavornet.org).

2.5 Statistical analysis

All analyses were performed in triplicate and means were evaluated by two-way analysis of variance (ANOVA) followed by Tukey's test and statistical differences were considered when $p < 0.05$. Principal Component Analysis (PCA) analyzes were performed using by auto-scaled means of samples' triplicates at software XLSAT (Addinsoft, Paris, France, version 2021.3.1) and plots using Microsoft Excel 2002 (MapInfo Corporation, Troy, NY, USA).

3 Results and discussion

3.1 Results of fermentation and physicochemical analyses

NAB (non-alcoholic beverages) and GFB (gluten-free beverages) are significant trends in the brewery sector and present a technological challenge to produce beverages with low or no alcohol content and/or gluten-free, with minimal changes to beer characteristics. Vacuum evaporation is the most economical process for producing NAB (Andrés-Iglesias et al., 2015), while the use of peptidases has emerged as an important biotechnological technique to produce GFB (Rubio-Flores & Serna-Saldivar, 2016).

Table 1 presents the results for color, original and apparent extract, alcohol content, and gluten amount in the trials performed. Fermentation with *S. cerevisiae* yeast in barley malt in the presence of prolyl endopeptidase (BScP) produced the highest alcohol content (3.30% ± 0.65) ($p < 0.05$). The use of a rotary evaporator with vacuum in *S. cerevisiae* beer fermentation was an attempt to reduce the alcohol content without significantly altering the beer's aroma. This technique was effective in reducing alcohol content in rice-fermented beer but not in malted beer. *S. ludwigii* yeast was used for fermentation in barley malt in the presence of prolyl endopeptidase (BSlP) and only 1.81% ± 0.31 ($p < 0.05$) ethanol was observed. When both yeasts were used for fermentation in malted rice, the alcohol content did not differ ($p > 0.05$) among samples. Brazilian regulation (Brasil, 2019) establishes that an NAB may contain a maximum of 0.5% alcohol; thus, none of the treatments proposed in this work met this criterion. On the other hand, the same regulation allows up to 2% alcohol to classify the beer as low-alcoholic (Brasil, 2019). Therefore, except for the BScP treatment, results show the adequacy of the trials to produce a low-alcoholic beverage. De Francesco et al. (2015) produced a NAB (alcohol content <1%) using *S. ludwigii* strains and credited this ability to the yeast's inability to ferment maltose. Similar results were found by Callejo et al. (2019), who observed that beers produced with *S. ludwigii* had lower alcohol concentrations than those with *S. cerevisiae*, *S. pombe*, and *Lachancea thermotolerans*. The inability to ferment maltose by *S. ludwigii* leads to low alcohol content and a sweet taste in beers (Adamenko et al., 2020).

Regarding gluten content, amounts of the peptide were found within the barley samples (BScP and BSlP) and did not differ between them ($p > 0.05$). BScP had 23.85 ± 4.42 mg/L of gluten, and BSlP had 8.56 ± 12.11 mg/L. The detection limit of the immunoassay method was 10 mg/L, according to the manufacturer. Samples made of malted rice were not evaluated since there is no prior assumption to be presented (none of the ingredients contain gluten). Worldwide, legal standards for GFB differ for instance, in the European Union and the USA, there is a limit of 20 parts per million of gluten (ppm, mg/L) for a brewery product to be considered "gluten-free," while in Australia, only beers with no detectable gluten can be labeled as gluten-

free (Rubio-Flores & Serna-Saldivar, 2016). In the European Union, foodstuffs for people intolerant to gluten that contain a level of gluten not exceeding 100 mg/kg may bear the term "very low gluten" (European Union, 2009). In the present work, it was possible to produce a low-gluten beer with barley malts using only commercial prolyl endopeptidase, but not a GFB.

Density parameters showed the highest values of OG in BScP and BSlP treatments, and the lowest ($p < 0.05$) in RSc, while FG values were higher in BSlP, RSc, and RSl treatments, which did not differ among them ($p > 0.05$), and the lowest density parameter was found in the BScP treatment ($p < 0.05$). Higher OG values may yield higher amounts of alcohol, which is critical for NAB production (Callejo et al., 2019). Ceppi & Brenna (2010) produced a beer-like beverage with 100% rice malt and reported reduced brewhouse yields due to incomplete saccharification and obtained beers with acceptable FG (3.1-3.6 °P) and alcohol content (3.6-4.5%).

Table 1. Physicochemical parameters of beers evaluated.

Parameters	BScP	BSlP	RSc	RSl
Alcohol (% v/v)	3.30 ± 0.65^{a}	1.81 ± 0.31^{b}	1.09 ± 0.36^{b}	0.88 ± 0.47^{b}
Gluten (mg/L)	23.85 ± 4.42^{a}	8.56 ± 12.11^{a}	n.a.	n.a.
OG (°P)	9.82 ± 0.04^{a}	8.69 ± 0.04^{ab}	5.74 ± 0.90^{c}	7.11 ± 0.61^{b}
FG (°P)	2.45 ± 0.18^{b}	5.24 ± 0.11^{a}	5.27 ± 0.32^{a}	5.39 ± 0.29^{a}
Color (EBC)	13.87 ± 0.78^{b}	7.68 ± 0.46^{c}	25.56 ± 6.67^{a}	21.49 ± 0.30^{a}
Color (RSC)	7.04 ± 0.40^{b}	3.90 ± 0.23^{c}	12.98 ± 3.39^{a}	10.91 ± 0.15^{a}

^{a,b,c}Different superscript letters indicate significant differences among columns at 5% of significance by the Tukey test. BScP: fermentation with *S. cerevisiae* yeast in barley malt in the presence of Prolyl endopeptidase; BSlP: fermentation with *S. ludwigii* yeast in barley malt in the presence of Prolyl endopeptidase; RSc: fermentation of *S. cerevisiae* yeast in malted rice; RSl: fermentation of *S. ludwigii* yeast in malted rice; OG: Original Gravity; FG: Final Gravity (FG); n.a.: not analyzed.

Color analysis showed significant differences between barley and rice malt beers in terms of EBC and RSC values ($p < 0.05$), with RSc and RSl presenting higher values ($p < 0.05$) than BScP and BSlP. When *S. cerevisiae* was used, higher EBC and RSC values ($p < 0.05$) were observed compared to trials using *S. ludwigii*. Low EBC values indicate pale beers, while higher EBC values describe darker beers (Koren et al., 2020). Color parameters did not differ ($p > 0.05$) between both trials with malted rice. It is important to point out the differences in malt composition between BScP and BSlP due to the different needs of the yeasts to perform beer production, according to previous works at the local brewery where the experiments were conducted. Bagatini et al. (2019) compared brewers' spent grain from beverages produced with just Pilsen malt and a combination of Pilsen, Melano, Carared (EBC 20), and Cara1 20 malts, observing that the addition of these compounds resulted in darker samples, although color parameters of yellowness and redness did not differ.

3.2 Results of volatile compounds

Table 2 shows the volatile compounds found among the samples, with 77 different compounds identified, belonging to more than five different chemical classes (acids, alcohols, aldehydes, esters, ketones, and other classes).

Table 2. Amounts of volatile compounds detected in beers produced with *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, barley and rice malts.

Volatile compounds	LRI_{exp}	LRI_{lit}	Aroma	BScP	BSlP	RSc	RSl
				Acids (µg/L)			
Isobutyric acic	1563	1563	Rancid, butter, cheese[1]	5.766 ± 1.052^{b}	7.089 ± 2.453^{b}	18.552 ± 5.87^{a}	15.485 ± 2.972^{a}
Butyric acid	1625	1642(a)	Cheese[1]	2.228 ± 0.207^{b}	1.555 ± 0.329^{c}	3.534 ± 1.520^{b}	10.115 ± 0.902^{a}
Isovaleric acid	1666	1665	Sweat, acid, rancid[2]	12.183 ± 0.660^{c}	9.2431 ± 3.869^{c}	87.823 ± 14.466^{a}	36.3000 ± 5.521^{b}
Caproic acid	1846	1829	Sweat[1,3,4]	51.221 ± 6.453^{b}	18.594 ± 4.932^{c}	17.933 ± 14.148^{c}	130.797 ± 29.273^{a}
5-methyl hexanoic acid	1907	1914		2.368 ± 0.411^{b}	2.4392 ± 0.684^{b}	1.118 ± 0.685^{c}	3.657 ± 0.504^{a}

Table 2. Continued…

Volatile compounds	LRI_{exp}	LRI_{lit}	Aroma	BScP	BSlP	RSc	RSl
				Acids (µg/L)			
Heptanoic acid	1955	1952		18.867 ± 2.673^{a}	13.459 ± 6.037^{ab}	6.010 ± 3.850^{b}	27.1860 ± 6.430^{a}
Caprylic acid	2063	2083	Sweat, cheese[1,3]	106.527 ± 32.632^{a}	30.741 ± 13.102^{b}	15.037 ± 15.037^{b}	71.753 ± 12.014^{a}
Nonanoic acid	>2300	2202	Green, fat[5]	3.843 ± 2.334^{a}	2.378 ± 0.881^{a}	1.096 ± 0.438^{a}	1.898 ± 0.449^{a}
Capric acid	>2300	2361	Rancid, fat[1]	3.812 ± 2.492^{a}	1.732 ± 0.889^{ab}	0.597 ± 0.271^{b}	1.546 ± 0.420^{b}
∑ Acids				206.814	87.229	151.700	298.735
				Alcohol (µg/L)			
2-methyl 3-buten-2-ol	1037	1030		14.264 ± 7.459^{b}	3.124 ± 1.967^{c}	10.672 ± 3.571^{b}	37.218 ± 6.361^{a}
Isobutanol	1093	1099	Wine, solvent, bitter[1]	83.839 ± 38.261^{a}	33.641 ± 23.481^{b}	25.919 ± 23.325^{b}	20.678 ± 6.472^{b}
2-methyl-1-butanol	1205	1208	Malt[1]	67.693 ± 23.423^{a}	46.257 ± 30.168^{a}	29.247 ± 6.169^{b}	25.719 ± 9.258^{b}
Isoamyl alcohol	1208	1215(a)	Beer, malt[6]	199.195 ± 61.503^{a}	126.346 ± 68.625^{ab}	77.963 ± 36.721^{b}	61.395 ± 16.556^{b}
Amyl alcohol	1249	1272		0.606 ± 0.100^{c}	1.121 ± 0.420^{b}	1.777 ± 0.479^{a}	1.392 ± 0.201^{ab}
Carpylic alcohol	1264	1265		-	0.091 ± 0.014^{a}	-	0.905 ± 0.068^{a}
3-methyl-2-buten-1-ol	1324	1324		0.527 ± 0.120^{b}	0.659 ± 0.47^{b}1	1.652 ± 1.393^{a}	1.699 ± 0.293^{a}
1-Hexanol	1353	1360	Resin, flower, green[1,3,7]	12.916 ± 8.741^{a}	19.771 ± 5.544^{a}	20.859 ± 4.124^{a}	12.517 ± 2.462^{a}
3-Hexen-1-ol	1382	1386	Moss, fresh[8]	4.974 ± 2.090^{a}	8.003 ± 3.023^{a}	5.625 ± 4.475^{a}	4.474 ± 0.591^{a}
3-Octanol	1395	1388	Moss, nut, mushroom[5,6]	40.700 ± 0.000^{a}	41.500 ± 0.005^{a}	42.702 ± 0.004^{a}	40.704 ± 0.001^{a}
1-Octen-3-ol	1449	1448		41.528 ± 9.827^{a}	29.872 ± 4.154^{ab}	19.543 ± 11.348^{b}	28.255 ± 2.807^{ab}
1-Heptanol	1456	1467	Chemical, green[9]	5.441 ± 2.530^{a}	6.597 ± 1.500^{a}	8.446 ± 3.848^{a}	5.134 ± 1.279^{a}
2-ethyl-1-hexanol	1489	1487	Rose, green[7]	8.402 ± 1.059^{b}	5.249 ± 0.418^{c}	27.775 ± 9.807^{a}	3.880 ± 0.513^{d}
2-Decanol	1523			5.839 ± 1.599^{b}	13.380 ± 2.503^{a}	4.623 ± 0.324^{b}	4.623 ± 1.034^{b}
1-Octanol	1560	1553	Chemical, metal, burnt[6,7]	4.663 ± 1.741^{a}	4.623 ± 0.569^{a}	4.474 ± 2.484^{a}	4.903 ± 1.148^{a}
2-ethoxy cyclohexanol	1526			3.174 ± 0.425^{ab}	2.791 ± 0.304^{b}	2.196 ± 0.792^{b}	4.570 ± 0.330^{a}
2,3-Butanediol	1592	1583	Fruit[10]	-	-	111.881 ± 93.694^{a}	14.671 ± 1.818^{b}
Benzene methanol<alpha-methyl->	1737			-	0.202 ± 0.165^{b}	5.916 ± 7.841^{a}	4.084 ± 3.384^{a}
Benzyl Alcohol	1810	1865	Sweet, flower[3]	2.425 ± 1.153^{a}	1.817 ± 0.496^{a}	1.736 ± 0.975^{a}	1.852 ± 0.256^{a}
Phenylethyl Alcohol	1921	1934(a)	Rose, honey[4]	2,814.596 ± 277.022^{a}	237.735 ± 137.924^{bc}	137.100 ± 78.396^{c}	406.661 ± 112.172^{b}
∑Alcohols				3,580.142	816.001	656.396	931.834
				Aldehydes (µg/L)			
Acetaldehyde	<700	718(a)	Green apple[10]	2.638 ± 0.383^{b}	3.827 ± 0.361^{ab}	8.217 ± 5.740^{a}	5.596 ± 1.766^{a}
Hexanal	1050	1084	Grass, tallow, fat[11]	2.102 ± 0.480^{a}	0.232 ± 0.134^{c}	0.495 ± 0.190^{b}	1.429 ± 0.904^{a}
Benzaldehyde	1518	1495	Almond, burnt sugar[11]	5.717 ± 2.606^{a}	2.912 ± 0.099^{b}	3.307 ± 2.697^{ab}	7.400 ± 1.429^{a}
∑Aldehydes				10.457	6.972	12.019	14.426
				Ester (µg/L)			
Ethyl acetate	864	907	Pineapple[6]	47.878 ± 33.553^{ab}	54.583 ± 10.283^{ab}	71.643 ± 4.995^{a}	40.995 ± 3.836^{b}
Ehtyl butyrate	1028	1042	Apple[1,7]	1.263 ± 0.433^{bc}	1.066 ± 0.135^{c}	2.009 ± 0.280^{a}	1.519 ± 0.037^{b}
Isobutyl isobuthyrate	1083			0.877 ± 0.301^{b}	0.876 ± 0.285^{b}	0.326 ± 0.074^{c}	0.983 ± 0.109^{a}
Isoamyl acetate	1114	1117	Banana[1]	42.480 ± 4.050^{ab}	36.549 ± 4.746^{b}	40.533 ± 3.111^{ab}	50.665 ± 6.598^{a}
1-Butanol, 3-methyl-, propanoate	1181	1180		1.459 ± 0.382^{b}	1.370 ± 1.006^{b}	0.365 ± 0.270^{c}	2.498 ± 0.394^{a}
2-methyl isobutyrate	1190	1192		2.374 ± 0.735^{a}	1.409 ± 0.584^{a}	0.663 ± 0.131^{b}	2.099 ± 0.275^{a}
Ethyl hexanoate	1226	1229	Jelly palm[12]	1.068 ± 0.128^{b}	-	-	1.664 ± 0.367^{a}
methyl 4-methylenehexanoate	1326	1324		0.855 ± 0.343^{ab}	0.623 ± 0.356^{b}	0.107 ± 0.009^{c}	0.933 ± 0.148^{a}
Lactic acid, ethyl ester	1341	1340		6.746 ± 4.356^{a}	6.3156 ± 2.275^{a}	0.624 ± 0.320^{b}	9.733 ± 1.241^{a}
Ethyl caprylate	1429	1436	Fruit, fat[7]	1.055 ± 0.426^{a}	0.220 ± 0.382^{b}	-	1.560 ± 0.479^{a}
Ammonium acetate	1447	1445		24.071 ± 4.720^{b}	21.816 ± 5.042^{b}	69.291 ± 74.929^{ab}	80.348 ± 57.571^{a}
Benzenemethanol, .alpha.-methyl-, acetate	1698	1692		8.889 ± 4.540^{a}	0.858 ± 0.145^{a}	12.206 ± 9.987^{a}	14.216 ± 13.673^{a}
Citral	1739	1735		4.255 ± 0.372^{ab}	1.880 ± 0.533^{b}	2.458 ± 2.131^{b}	6.997 ± 1.883^{a}
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	2013	2007		4.364 ± 0.663^{a}	3.637 ± 1.048^{a}	1.446 ± 0.652^{b}	4.144 ± 0.763^{a}
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	2014	2015		26.898 ± 4.616^{a}	8.099 ± 3.006^{b}	12.273 ± 5.866^{ab}	8.935 ± 4.784^{b}

Table 2. Continued…

Volatile compounds	LRI_{exp}	LRI_{lit}	Aroma	BScP	BSlP	RSc	RSl
				Acids (µg/L)			
4-Heptenoic acid, ethyl ester, (E)-	2014	2013		1.636 ± 0.258^{a}	1.553 ± 0.730^{a}	0.310 ± 0.017^{b}	1.016 ± 0.414^{a}
Benzyl benzoate	>2300	2071	Balsamic, oil, herb[2]	0.920 ± 0.194^{a}	0.389 ± 0.091^{b}	0.351 ± 0.343^{b}	1.969 ± 0.526^{a}
∑Esters				177.087	141.245	214.606	230.274
				Ketones(µg/L)			
Acetone				3.537 ± 1.763^{b}	1.879 ± 0.226^{c}	3.964 ± 2.259^{b}	6.289 ± 1.249^{a}
Methyl Isobutyl Ketone	1007	1002		1.812 ± 1.333^{b}	2.125 ± 0.307^{b}	3.011 ± 2.259^{ab}	5.011 ± 0.686 ^{a}
2-Butanone, 3-hydroxy-	1243	1245		-	0.655 ± 0.121^{a}	18.992 ± 18.721a	8.710 ± 2.227^{a}
Sulcatone	1334	1330		13.852 ± 3.174^{a}	6.132 ± 0.957^{b}	5.094 ± 4.291^{b}	16.783 ± 1.249^{a}
Acetophenone	1652	1645	Must, flower, almond[5]	2.933 ± 1.509^{a}	0.156 ± 0.027^{b}	2.192 ± 1.556^{a}	2.370 ± 1.660^{a}
∑Ketones				22.135	10.948	33.254	39.164
				Terpenes(µg/L)			
Linalool	1549	1537	Flower, lavender[1]	241.394 ± 52.100^{a}	224.013 ± 11.075^{a}	130.913 ± 114.949^{a}	253.829 ± 44.946^{a}
Beta-myrcene	1151	1145	Balsamic, must, spice[10]	2.069 ± 1.002^{a}	1.256 ± 0.654^{a}	0.449 ± 0.002^{b}	1.270 ± 0.390^{a}
Neral	1687	1667	Lemon[1,6]	1.586 ± 0.229^{b}	0.589 ± 0.111^{c}	0.689 ± 0.596^{c}	3.286 ± 0.880^{a}
3,7-octadien-2-ol, 2-methyl-6-methylene, (e)	1632	1637		25.517 ± 1.927^{a}	20.072 ± 1.948^{a}	17.521 ± 13.714^{a}	34.494 ± 7.235^{a}
Hydroxycitronellol	1474	1479	Floral[6]	3.612 ± 0.307^{a}	3.998 ± 0.528^{a}	4.024 ± 5.501^{a}	3.051 ± 0.042^{a}
Terpinen-4-ol	1603.016	1592	Turpentine, nutmeg, must[6]	4.038 ± 0.736^{a}	3.677 ± 0.119^{a}	3.054 ± 1.179^{a}	4.156 ± 0.693^{a}
Isomycenol	1682.688	1686		6.298 ± 0.966^{a}	5.147 ± 0.941^{a}	4.832 ± 1.593^{a}	7.652 ± 0.608^{a}
p-menth-1-en-8-ol	1703.061	1700		5.342 ± 0.819^{b}	11.718 ± 1.958^{a}	5.671 ± 2.267^{b}	6.765 ± 2.909^{b}
2,6-dimethyl 1,5,7-octatrien-3-ol	1749.102	1752		4.963 ± 0.755^{b}	2.426 ± 0.165^{b}	3.043 ± 2.219^{b}	6.844 ± 2.227^{a}
Citronelol	1771.194	1770	Roses, flower[5,6]	13.866 ± 14.380^{b}	61.429 ± 40.142^{a}	2.854 ± 4.027^{b}	5.353 ± 0.997^{b}
Geraniol	1805	1802		10.218 ± 1.662^{a}	15.653 ± 1.663^{a}	8.547 ± 8.194^{a}	9.070 ± 1.108^{a}
Nerol	1856	1852		221.024 ± 26.374^{a}	129.974 ± 45.523^{ab}	86.268 ± 74.138^{b}	203.611 ± 33.612^{a}
∑Terpenes				269.125	234.022	118.293	246.502
				Others (µg/L)			
Ethane, 1,1-diethoxy-	881			41.449 ± 22.256^{b}	21.522 ± 6.841^{b}	111.791 ± 59.112^{a}	69.613 ± 12.401^{ab}
Propane, 1,1-diethoxy-2-methyl-	938			-	0.705 ± 0.119^{c}	1.281 ± 0.068^{b}	1.941 ± 0.252^{a}
1,3-cyclohexadiene, 5-(3-buten-1-yl)-	1209			0.897 ± 0.040^{b}	1.450 ± 0.281^{ab}	0.435 ± 0.364^{c}	2.019 ± 0.453^{a}
4h-cyclopenta[c]furan, hexahydro-1,1-dimethyl-4-methylene-	-			4.844 ± 1.066b^{a}	4.426 ± 0.549^{ab}	2.136 ± 1.308^{b}	5.445 ± 0.680^{a}
Furanoid	1442	1449	Flower[10]	1.590 ± 0.391^{a}	0.913 ± 0.224^{a}	5.085 ± 4.854^{a}	3.251 ± 0.445^{a}
2-furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, trans-	1472			0.837 ± 0.086^{a}	0.371 ± 0.046^{a}	3.972 ± 4.875^{a}	1.768 ± 0.277^{a}
Phenol	2010	1479	Phenol[10]	0.428 ± 0.082^{c}	0.242 ± 0.047^{d}	5.058 ± 3.914^{a}	1.646 ± 0.289^{b}
Ethene, ethoxy-	-			2.326 ± 0.293^{b}	1.275 ± 0.573^{b}	4.010 ± 1.452^{a}	2.400 ± 0.392
Butanimidamide	1151	1142		-	1.406 ± 0.534^{a}	0.742 ± 0.073^{b}	0.768 ± 0.075^{b}
Dihydro-5-pentyl-2(3h)-furanone	2048	2047(a)	Candy floss, caramel[11]	3.711 ± 0.029^{b}	1.246 ± 0.248^{c}	2.003 ± 1.093^{c}	9.538 ± 1.704^{a}
Ethanone, 1-(1h-pyrrol-2-yl)-	1984	1977		1.007 ± 0.257^{b}	0.273 ± 0.023^{d}	0.315 ± 0.017^{c}	1.779 ± 0.392^{a}

Values given are mean areas following standard deviations from triplicates. $^{a.b.c,d}$Different superscript letters indicate statistical difference among columns at 5% of significance by Tukey test. LRI_{exp}: linear retention indexes calculated; LRI_{lit}: linear retention indexes in the literature; - not identified based on the column used; [1]Rychlik et al. (1998); [2]Ong & Acree (1999); [3]Adedeji et al. (1991); [4]Miranda-Lopez et al. (1992); [5]Jirovetz et al. (2002); [6]Chung et al. (1993); [7]Moio et al. (1993); [8]Pennarun et al. (2002); [9]Tamura et al. (1995); [10]Tan & Siebert (2004); [11]Triqui & Reineccius (1995); [12]Bernardi et al. (2014).

Volatile acids are important aromatic compounds in fermented beverages, as they can contribute to off-flavors such as rancid, sweat, and cheesy notes (Rychlik et al., 1998; Adedeji et al., 1991). Isobutyric, butyric, and isovaleric acids, which present rancid/cheesy notes (Rychlik et al., 1998; Ong & Acree, 1999), were found in higher concentrations ($p < 0.05$) when malted rice was used as a carbohydrate source (RSc and RSl). Caprylic acid, with an odor threshold of 500 mg/L (Moreno et al., 2005), was in lower concentration than the perception limit but was higher in BScP and RSl than BSlP and RSc ($p < 0.05$). Amounts of isobutyric and isovaleric acids did not differ between BScP and BSlP ($p > 0.05$), whereas BScP had higher values ($p < 0.05$) for butyric and caproic acids than BSlP. RSl also had the highest concentration of caproic acid ($p < 0.05$), which contributes to sweat/cheesy aromas (Miranda-Lopez et al., 1992; Rychlik et al., 1998). When barley was used as malt and fermented with S. cerevisiae, even after vacuum evaporation, high amounts of capric acid (rancid/fat notes) (Rychlik et al., 1998) were observed compared to samples with malted rice ($p < 0.05$). Caproic acid (also known as hexanoic acid, which presents sweat notes) has an odor threshold of 420 mg/L (Adedeji et al., 1991; Miranda-Lopez et al., 1992; Rychlik et al., 1998); the samples presented lower amounts than its perception limit (Table 2).

Volatile alcohols are the most abundant components in beers and an important source of flavor (Callejo et al., 2019). The main sources of alcohol in wine products are derived from yeast and bacterial metabolism by decarboxylation of ketoacids, intermediates of biochemical changes in leucine, isoleucine, valine, and threonine. They can also be formed by lipid oxidation metabolites and glycosylated precursors (Hazelwood et al., 2008). The highest concentration (3,580.142 ± 402.357 µg/L) of alcohols was found in BScP ($p < 0.05$) and the lowest in RSc (656.396 ± 62.640 µg/L). Samples BSlP and RSl did not differ in total volatile alcohol content ($p > 0.05$). Mayer et al. (2016) observed that 100% rice beer presented greater amounts of higher alcohols than barley malt bottom-fermented beers due to the reduced concentration of amino acids and relatively high pitching temperature used. Liu et al. (2011) observed that beers fermented with *S. ludwigii* had low production of esters and higher alcohols. The amount of isobutanol, which has a wine/solvent aroma (Rychlik et al., 1998), was higher than the odor threshold (16 mg/L) (Moreno et al., 2005) and was the highest ($p < 0.05$) in BScP, while in malted rice samples (RSc and RSl) it did not differ ($p > 0.05$). A similar pattern was observed for 2-methyl-1-butanol and isoamyl alcohol (also known as 3-methyl-1-butanol), which have malt and beer malt notes, respectively (Chung et al., 1993; Rychlik et al., 1998), although BScP and BSlP samples did not differ ($p > 0.05$) for both compounds. Isoamyl alcohol, with an odor threshold of 30 mg/L (Peinado et al., 2004), was present in higher amounts than the aroma perception limit. 1-Hexanol, 3-hexen-1-ol, 1-octanol, 1-heptanol, and benzyl alcohol, which have respectively resin/flower/green, moss/fresh, chemical/green, chemical/metal/burnt, sweet/flowers, and turpentine/nutmeg/must notes (Adedeji et al., 1991; Chung et al., 1993; Rychlik et al., 1998; Pennarun et al., 2002; Jirovetz et al., 2002), did not differ among samples ($p > 0.05$). Samples had higher amounts of 1-octanol than its odor threshold of 1 mg/L (van Gemert, 2011). 1-Hexanol, 3-hexen-1-ol, and benzyl alcohol were in lower concentrations than their odor thresholds (800, 400, and 200 mg/L, respectively) (Moreno et al., 2005; Zalacain et al., 2007). It is well documented that several aroma compounds are lost during NAB production through thermal processes and that some unpleasant flavors, such as bready, worty, or caramel notes, can form (Catarino & Mendes, 2011; Lehnert et al., 2009; Sohrabvandi et al., 2010; Andrés-Iglesias et al., 2015). 2,3-Butanediol, an important volatile compound in fermented foods, was in lower amounts than its odor threshold (668 mg/L) (Moreno et al., 2005) and was present only in samples produced with malted rice, with higher content observed when *S. cerevisiae* was used ($p < 0.05$). Additionally, the use of *S. cerevisiae* led to a higher content of 2-ethyl-1-hexanol (rose/green aroma) (Moio et al., 1993) than the use of *S. ludwigii* yeast in both treatments ($p < 0.05$). Phenylethyl alcohol, an important volatile compound in alcoholic beverages contributing to a rose/honey aroma, was highest ($p < 0.05$) in BScP and lowest ($p < 0.05$) in RSc, with all samples presenting higher amounts than the perception limit of 10 mg/L (Moreno et al., 2005).

Aldehydes are important flavor-active compounds in beers and are usually considered negative attributes of beer flavor (De Francesco et al., 2015). Lower concentrations ($p < 0.05$) of these compounds were observed in BSlP samples, which did not differ from RSc ($p > 0.05$), consistent with Mayer et al. (2016), who observed aldehyde

amounts in rice beer compared to barley-based brewery. Acetaldehyde, which contributes to green/apple notes (Tan & Siebert, 2004), was in higher amounts ($p < 0.05$) in BSlP, RSc, and RSl samples. Acetaldehyde, formed as a metabolic branch point in the pathway from carbohydrate to ethanol, has levels directly related to ethanol content (De Francesco et al., 2015) and an odor threshold of 10 mg/L. Hexanal (with grass/tallow/fat odor) was in higher amounts ($p < 0.05$) in BScP and RSl. Also, BScP, RSc, and RSl did not differ ($p > 0.05$) in benzaldehyde content, which has almond/burnt sugar notes (Triqui & Reineccius, 1995) and higher values than BSlP. Benzaldehyde amounts were close to the odor threshold (5 mg/L) (Moreno et al., 2005; van Gemert, 2011) for all samples.

Esters are critical compounds in fermented foods as they contribute flowery and fruity notes to the product and have low thresholds. They are formed by intracellular metabolism of fermenting yeast cells. Since they are lipid-soluble, ethyl esters can diffuse through the cellular membrane into the fermenting medium (Saerens et al., 2008). Higher amounts of esters were found in beers produced with malted rice ($p < 0.05$) and did not differ between samples produced with the same malt ($p > 0.05$) (Table 2). Mayer et al. (2016) also observed higher ester amounts in rice beer compared to barley-based breweries. Among those identified within the samples with well-documented aromas, ethyl acetate, which has pineapple notes (Chung et al., 1993), was in higher amounts than the odor threshold (7.5 mg/L) (Moreno et al., 2005) and did not differ ($p > 0.05$) between BScP and BSlP, while higher amounts of the compound were observed in RSc than RSl ($p < 0.05$). Isoamyl acetate, which has banana notes (Rychlik et al., 1998) and was in higher amounts than the threshold (30 mg/L) (Moreno et al., 2005), did not differ between BScP and BSlP and RSc and RSl samples, although higher amounts were observed when *S. ludwigii* yeast was present in malted rice ($p < 0.05$) compared to when barley malt was used. Ethyl caprylate (also called ethyl octanoate) (in lower amounts than the odor threshold of 5 mg/L) (Peinado et al., 2004) (fruit/fat aroma) (Moio et al., 1993) and benzyl benzoate (balsamic/oil/herb notes) (Adedeji et al., 1991) were present in higher amounts in BScP and RSl samples and lowest in BSlP ($p < 0.05$), although ethyl caprylate was not detected in RSc samples, and benzyl benzoate amounts did not differ ($p > 0.05$) between BSlP and RSc samples. Ethyl hexanoate, which has a jelly palm odor (Bernardi et al., 2014), was not detected in BSlP and RSc samples and was found below the sensory threshold (5 mg/L) (Moreno et al., 2005) in RSl compared to BScP ($p < 0.05$). De Francesco et al. (2015) observed that *S. ludwigii* strains produced high ester content and a lower amount of diacetyl compared to previous reports. Callejo et al. (2019) attributed higher concentrations of ethyl acetate, isobutyl acetate, isoamyl acetate, and 2-phenylethyl acetate to high OG values. Beers produced by *S. ludwigii* have been reported to have high amounts of diacetyl, although it did not affect the sensory profile of the product (Callejo et al., 2019).

Among ketones, acetone was in the highest amount in RSl samples and lowest in BSl samples ($p < 0.05$). For acetophenone, which has must/flower/almond aroma (Jirovetz et al., 2002), the highest amounts were observed in BScP, RSc, and RSl samples, and the lowest amount in BSlP ($p < 0.05$). Linear ketones are formed by free fatty acid oxidation (Narváez-Rivas et al., 2012); other ketones such as methyl ketones can form through Maillard reactions (Pérez-Santaescolástica et al., 2018), but they can also be produced by microbial carbohydrate metabolism (Corino et al., 2003; Petričević et al., 2018).

Terpenes are usually related to hops added in beers. Hydroxycitronellol, terpinen-4-ol, isomycenol, and geraniol were found in all three trials and did not differ ($p > 0.05$). p-menth-1-en-8-ol and citronellol were in higher amounts in BSlP ($p < 0.05$) and did not differ among the other treatments ($p > 0.05$). RSc had lower ($p < 0.05$) amounts of nerol, although it did not differ from BSlP ($p > 0.05$). Linalool, which has flower and flower/lavender notes and an odor perception threshold of 15 mg/L (Rychlik et al., 1998), did not differ among samples ($p > 0.05$), with amounts higher than this value in all samples. Neral, with lemon notes (Chung et al., 1993; Rychlik et al., 1998), was in the highest amount ($p < 0.05$) in RSl samples and the lowest in BSlP and RSc, which did not differ ($p > 0.05$). Beta-myrcene, which has balsamic/must/spice notes, was in the highest amount in BScP, BSlP, and RSl samples and the lowest amount in RSc.

Among other compounds, furanoid (flower notes) (Rychlik et al., 1998) did not differ among samples ($p > 0.05$). Phenol amounts were higher ($p < 0.05$) in malted rice samples, being higher in RSc than RSl ($p < 0.05$) and higher

in BScP than BSlP ($p < 0.05$). Dihydro-5-pentyl-2(3h)-furanone, which has a candy/caramel aroma (Zehentbauer & Reineccius, 2002) was in higher concentration RSl, followed by BScP, BSlP and RSc.

To evaluate similar patterns among samples and visualize their profiles considering all parameters, PCA analysis was performed. Results are shown in Figure 1, and dimension reduction explained 84.23% of the variance (49.86% by PC1 and 34.37% by PC2). PCA distinguished all samples by their volatile compound amounts. Multivariate analysis by Callejo et al. (2019) also differentiated beers produced by *S. cerevisiae* and *S. ludwigii*.

Figure 2 shows the PCA score plots results for BScP (*S. cerevisiae* yeast in barley malt in the presence of Prolyl endopeptidase); BSlP (*S. ludwigii* yeast in barley malt in the presence of Prolyl endopeptidase); RSc (*S. cerevisiae* yeast in malted rice); and RSl (*S. ludwigii* yeast in malted rice).

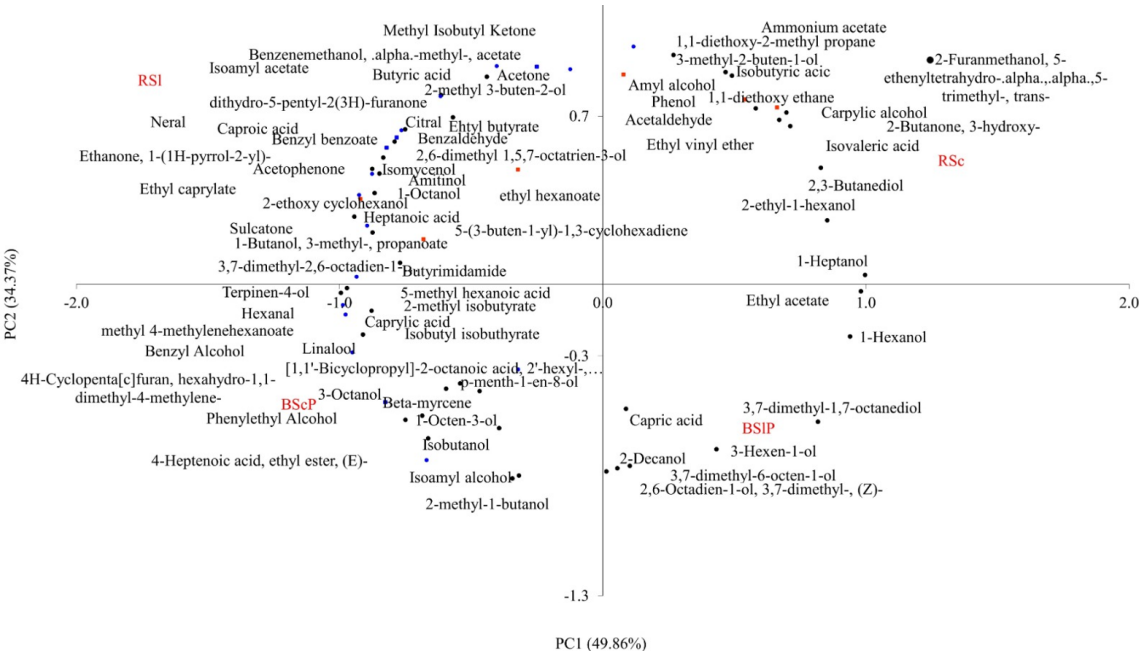

Figure 2. PCA score plots of BScP (*S. cerevisiae* yeast in barley malt in the presence of Prolyl endopeptidase); BSlP (*S. ludwigii* yeast in barley malt in the presence of Prolyl endopeptidase); RSc (*S. cerevisiae* yeast in malted rice) and RSl (*S. ludwigii* yeast in malted rice).

BScP beers (produced with barley malt, fermented with *S. cerevisiae* and in the presence of peptidase enzymes after vacuum evaporation) were associated to the presence of several volatile alcohols such as phenylethyl alcohol, 1-octen-3-ol, isobutanol, linalool, benzyl alcohol, isoamyl alcohol, and also to beta-myrcene, indicating that the sample highlighted for odor-active compounds with floral, malt/must and wine/solvent flavors. BSlP samples were associated to the presence of capric acid, 3-hexen-1-ol, 2-decanol, indicating that beers fermented with *S. ludwigii* and addition of peptidases evaporation highlights for moss/fresh, rancid/fat flavor, which may not be considered pleasant odors. Hidroxicitronelol and citronelol present flowery notes and comes probably due hops.

RSc was associated to the presence of 2,3-butanediol, isovaleric acid, ethyl vinyl ether and 2-ethyl-1-hexanol and 2-furanmethanol, 5-ethenyltetrahydro-alpha.,.alpha.,5-trimethyl-, trans- leading to rose/green, fruity and acid flavors. RSl was placed close to isoamyl acetate, citral, neral, amitinol, benzyl benzoate, caproic acid, leading to sweat, banana, lemon, balsamic/oil/herb flavor.

There is few information in literature exploring the combination of rice malt and *S. ludwigii* and utilization of polypeptidases on barley malt beers and more studies are necessary to optimize the process parameters in order to produce a high-quality beverage.

4 Conclusions

The use of *S. cerevisiae* combined with vacuum heat treatment or use of *S. ludwigii*, in malted rice or barley malt, resulted in low-alcoholic beers. The use of peptidases for gluten degradation in barley malt produced a beverage classified as a very low gluten product. Beer made with malted rice and fermented with *S. cerevisiae* had lower original gravity values, while beer fermented with *S. cerevisiae* in barley malt, with added peptidase and heat treatment, yielded the lowest final gravity values. Barley samples had the lowest color intensity. Different yeast and malt-base combinations led to varying volatile compound profiles. BScP beers were associated with several volatile alcohols, while BSlP samples had compounds that may not be considered pleasant odors. RSc beers had rose/green, fruity, and acidic flavors, whereas RSl beers had sweet, banana, lemon, balsamic/oily, and herbal flavors. More research is needed to explore these techniques for producing low/free gluten and non/low alcoholic beers to optimize their use on an industrial scale, ensuring maximum physiochemical and sensory quality.

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