UNOESC
Fazendo parte da sua vida

EVIDÊNCIA
BIOCIÊNCIAS, SAÚDE E INOVAÇÃO
evidence: bioscience, health and innovation

DOI: https://doi.org/10.18593/evid.37148

Seção: Biociências

Fabricação de cerveja artesanal utilizando Saccharomyces cerevisiae var. Boulardii como agente fermentador

Artisanal beer production using Saccharomyces cerevisiae var. boulardii as a fermenting agent

Bianca de Lima[1], Roberta Castro Martins[2], Claudia Eugênia Castro Bravo[1], Ellen Cristina Perin[3], Solange Teresinha Carpes[4], Ellen Porto Pinto[1]

[1] Engenharia de Alimentos, Universidade Tecnológica Federal do Paraná (UTFPR) – Francisco Beltrão, PR, Brasil; [2] Programa de Pós-Graduação em Engenharia de Biomateriais (PPGBIOMAT), Universidade Federal de Lavras (UFLA) – Lavras, MG, Brasil; [3] Departamento de Tecnologia e Ciência dos Alimentos (DTCA), Universidade Federal de Santa Maria (UFSM) – Santa Maria, RS, Brasil; [4] Programa de Pós-Graduação em Processos Químicos e Biotecnológicos (PPGQB), UTFPR – Pato Branco, PR, Brasil.

Lima, B. de
biadlima@outlook.com
https://orcid.org/0009-0006-7858-4394

Martins, R. C.
roberta.martins2@estudante.ufla.br
https://orcid.org/0000-0001-9325-5720

Bravo, C. E. C.
claudiacastro@utfpr.edu.br
https://orcid.org/0000-0002-5968-9864

Perin, E. C.
ellenperin@ufsm.br
https://orcid.org/0000-0002-0380-3576

Carpes, S. T.
carpes@utfpr.edu.br
https://orcid.org/0000-0001-8625-7795

Pinto, E. P.*
ellenporto@utfpr.edu.br
https://orcid.org/0000-0002-4230-2969

* Autora correspondente: Universidade Tecnológica Federal do Paraná, Campus Francisco Beltrão, PR, Rua Gelindo João Folador, n.º 2000, Bairro Novo Horizonte, CEP: 85602-863, Caixa Postal 135, Francisco Beltrão – PR, Brasil.

Resumo: O mercado de cervejas tornou-se altamente competitivo, destacando-se pela diferenciação de produtos e pela agregação de valor, com crescente interesse por itens que promovam benefícios à saúde. Neste contexto, a levedura *Saccharomyces cerevisiae var. boulardii*, originalmente isolada da lichia, apresenta-se como alternativa viável devido às suas propriedades probióticas amplamente utilizadas no tratamento de doenças gastrointestinais. Este estudo teve como objetivo produzir uma cerveja artesanal no estilo *Cream Ale* utilizando *S. boulardii* como agente fermentador, comparando suas características físico-químicas, compostos bioativos e atributos sensoriais com uma formulação padrão fermentada com *S. cerevisiae.* As análises realizadas incluíram pH, cor, amargor, teor alcoólico, extrato real, extrato primitivo, turbidez, compostos fenólicos totais, flavonoides totais, atividade antioxidante e avaliação sensorial por meio da técnica *Perfil Flash*. Os resultados indicaram que a formulação com *S. boulardii* apresentou menor conversão e atenuação, resultando em teor alcoólico inferior. Entretanto, essa formulação destacou-se pelo maior número de compostos fenólicos, associados a uma atividade antioxidante superior, fortemente correlacionada com os teores de flavonoides. Apesar das diferenças nas propriedades físico-químicas, as análises sensoriais não identificaram variações significativas entre as formulações. Esses achados sugerem que a utilização de *S. boulardii* pode agregar valor funcional às cervejas artesanais, embora ajustes no processo sejam necessários para melhorar o rendimento fermentativo e alcançar padrões comerciais. Estudos futuros devem explorar a viabilidade econômica, o impacto na conservação do produto e o desempenho da levedura em outros estilos de cerveja.
Palavras-chave: Bebidas fermentadas, Caracterização físico-química, Fermentação alcoólica, Leveduras.

Abstract: The beer market has become highly competitive, standing out for product differentiation and added value, with growing interest in items that promote health benefits. In this context, the yeast Saccharomyces cerevisiae var. boulardii, originally isolated from lychee, presents itself as a viable alternative due to its probiotic properties widely used in the treatment of gastrointestinal diseases. This study aimed to produce a craft beer in the Cream Ale style using S. boulardii as a fermenting agent, comparing its physicochemical characteristics, bioactive compounds, and sensory attributes with a standard formulation fermented with S. cerevisiae. The analyses included pH, color, bitterness, alcohol content, real extract, primitive extract, turbidity, total phenolic compounds, total flavonoids, antioxidant activity, and sensory evaluation using the Flash Profile technique. The results indicated that the formulation with S. boulardii showed lower conversion and attenuation, resulting in lower alcohol content. However, this formulation stood out for its higher number of phenolic compounds, associated with superior antioxidant activity, strongly correlated with flavonoid content. Despite differences in physicochemical properties, sensory analyses did not identify significant variations between the formulations. These findings suggest that the use of S. boulardii can add functional value to craft beers, although process adjustments are necessary to improve fermentative yield and achieve commercial standards. Future studies should explore economic viability, impact on product preservation, and yeast performance in other beer styles.
Keywords: Fermented beverages, Alcoholic fermentation, Yeasts, Physicochemical characterization.

Recebido: 23/04/2025 | **Aceito:** 20/08/2025 | **Publicado:** 22/07/2026
Editor: Marcos Freitas Cordeiro

INTRODUCTION

In 2020, there were already over 19 thousand registered breweries worldwide, with approximately 94% of them being craft breweries. Craft beer distinguishes itself primarily through the pursuit of unique aromas and flavors. Brazil is the 3rd largest consumer of beer in the world with an annual consumption of 14.3 billion liters, which represents 7% of world consumption, behind China and the United States, which represent 27% and 13% of world consumption, respectively. The exponential growth in the consumption of craft beers reflects the Brazilian consumer's increasing interest in acquiring unique tasting experiences and follows a global trend of valuing the artisanal. The perspective of the growth of craft breweries is high and has a positive scenario, only in the last decade there was a 91% increase in the number of local breweries and the country was one of the biggest investors in the brewing segment (Kirin Beer University Report, 2020; Sgorla, 2024; Toledo et al., 2018).

Concurrently the growth in beer consumption, there was also an increase in demand for foods with a health-oriented appeal, since the consumption of food and beverages is no longer driven solely by physiological needs, but also by aspects such as status, self-esteem, and well-being. Products perceived as healthier–such as organic items, “free-from” alternatives (e.g., gluten-free, lactose-free) and probiotics, even when not necessarily healthier from a clinical perspective these products are marketed and consumed based on their association with healthiness. Sustainable consumption and heightened awareness of health-related choices positively influence the consumer's sense of growth, pleasure, and emotional well-being (Issock et al., 2023).

Probiotics are live microorganisms that when administered correctly, have a beneficial effect on health, for which they need to survive the acid pH of the stomach to act together with the intestinal microbiota, fighting pathogens (Mendonça, 2021).

Saccharomyces cerevisiae var. boulardii was first isolated in 1920 by Henri Boulard from the skins of tropical lychee (*Litchi chinensis*) and mangosteen (*Garcinia mangostana L.*) in Indochina. After a French pharmaceutical company acquired the rights to the strain and its fermentation process in the 1950s, it was commercialized in lyophilized form as a probiotic. Today, its are added to supplements and foods, where they survive gastrointestinal transit, modulate the intestinal microbiota, and help prevent or treat various forms of diarrhea (Ansari et al., 2023; MacFarland & Li, 2025; Moreira Terhaag et al., 2025; Ryabtseva et al., 2022; Souza et al., 2022).

The yeast *S. cerevisiae* is the main fermenting agent in microbreweries, becoming a high cost for producers considering that most are imported from other countries. Even though it can be replicated and reused in up to three fermentation processes, the yeast is intolerant to inorganic acids and exhibits a high rate of mutant cell formation. Studies have shown that *S. boulardii* strains are more resistant to inorganic acids, the lowering of pH by ethanol conversion and other environmental variables during replication. Becoming a viable option for replicability in fermentation processes, reducing costs in microbreweries (Jenkins et al., 2009; Lazo-Vélez et al., 2018).

In this context, the objective of this work is to produce craft pure malt Cream Ale beer using *Saccharomyces cerevisiae* var. *boulardii* as fermenting agent and perform physical-chemical analysis, characterization of bioactive compounds and sensory analysis of the final product and statistically evaluate the differences between beer produced with *S. cerevisiae* for these parameters.

MATERIAL AND METHODS

Beer production

Two beer formulations were developed with variation only in the yeast used. In formulation A (standard) the fermenting agent was the yeast *S. cerevisiae* US-05 from the trademark Fermentis® (contains > 6 x 10^9 viable cells per gram) and in formulation B the fermenting agent used was the yeast *S. cerevisiae* var. *boulardii* obtained from the drug Floratil®, where each capsule contains 200 mg of lyophilized *Saccharomyces boulardii* – 17 (200 mg of lyophilized yeast contains at least 1 x 10^9 cells of *Saccharomyces boulardii* – 17).

The production of artisanal Cream Ale style beer followed the base formulation for the style with differences in the preinoculation and fermentation phases. Before inoculation, water and dry malt extract were solubilized to a density of 1.036. This solution was boiled for 5 min and cooled to 30 °C, then both yeasts were inoculated in different flasks and kept stirring for 24 hours (Mendonça, 2021). After inoculation into the must, fermentation was carried out at a temperature of 17 °C for 2 days to promote a controlled and clean initial fermentation, minimizing the formation of unwanted flavors and allowing yeast adaptation. Subsequently, the temperature was increased to 20 °C for 10 days to complete the attenuation efficiently, ensuring full sugar conversion and developing the light, smooth profile characteristic of Cream Ale. At the end of fermentation, the beer matured for another 10 days at temperatures of 0 °C.

After this period, the beer was transferred to another fermenter to avoid trub drag in the bottling, priming was added in the fermenter in the proportion of 7 $g.L^{-1}$ of beer to carbonate the beer. The bottling was made in amber glass bottles with a capacity of 300 mL, which remained at rest for another 30 days at an average room temperature of 16 ºC to complete the carbonation (2nd fermentation).

Physicochemical characterization of the beers produced

To carry out the physical-chemical characterization analyses, the beer had to be decarbonated. To remove CO2, the sample was stirred in a magnetic stirrer for 5 minutes (Bortoleto & Gomes, 2020; Instituto Adolfo Lutz [IAL], 2008). The determination of the pH occurred by direct reading in a pH meter (IAL, 2008).

The quantification of bitterness in International Bitterness Units (IBU) was performed using the EBC 9.8 spectrophotometric method (European Brewery Convention [EBC], 2020). The wavelength used in this method is 275 nm. This wavelength is specifically for measuring the concentration of alpha acids, compounds responsible for bitterness in beer.

For the characterization of beer in terms of color, a spectrophotometric method was used. The decarbonate samples were filtered through membrane filter paper, after which the absorbance was read at a wavelength of 430 nm, using ultra-pure water as a blank, in a spectrophotometer. To represent the results in EBC, the absorbance at 430 nm was multiplied by 25 (European Brewery Convention, 2005, as cited in Tozzeto, 2017).

Analysis of alcohol in volume and alcohol in weight were performed using methods 246/IV and 247/IV, respectively, from IAL (2008).

The real extract, apparent extract and primitive extract were determined following methods 249/IV, 250/IV and 251/IV of IAL (2008).

The turbidity determination followed the method described by Mafra (2018), using a turbidimeter previously adjusted to zero with distilled water, the samples were read in NTU. The value obtained in NTU was multiplied by 4 for transformation into EBC.

Characterization of bioactive compounds

To determine the total phenolic compounds (TFC), the Folin-Ciocalteau spectrophotometric method was used, based on Swain and Hillis (1959, as cited in Galli et al., 2016) Gallic acid was used as a standard and the results were expressed in mg of Gallic Acid equivalent (EAG) per mL of beer.

The methodology for determining flavonoids was described and adapted by Park et al. (1998) and Singh and Rathore (2018). Quercetin was used as a standard and the results were expressed in mg quercetin equivalent (EQC) per mL of beer.

The total antioxidant activity of the beverage was determined based on the DPPH method described by Brand-Williams, Cuvelier and Berset (1995, as cited in Galli et al., 2016). Trolox solution was used as a standard and data were presented as mmol of Trolox equivalent per mL of beer (mM TE. mL^{-1}).

For the determination of antioxidant activity by the FRAP method, the sample had to be diluted 5x. Then, 1 mL of sample was added to 4 mL of distilled water forming what is called an extract. 90 µL aliquots of extract were added to 270 µL of distilled water and 2.7 mL of FRAP reagent solution. This solution stayed at rest in a water bath at 37°C for 30 minutes away from light. After this time, the samples were read in a spectrophotometer at a wavelength of 595 nm. The standard curve was constructed using 2 mM ferrous sulfate as standard reagent, in concentrations ranging from 0.1 to 2 mM. The results were expressed in mmol Fe^{2+}.mL^{-1}.

The determination of antioxidant activity by ABTS (2,2' azinobis [3-ethylbenzothiazoline-6-sulfonic acid]) was based on the method developed by Re et al. (1999). The standard curve was constructed using Trolox solution as the standard reagent and therefore the results were expressed in mM TE.mL^{-1}.

Sensory analysis

The sensory analysis took place in three stages, initially there was the selection of the team, later the network method was used to survey attributes and finally the beers were evaluated using the descriptive Flash Profile analysis.

For the selection of judges, being a consumer of the beverage was mandatory. A total of 40 volunteers, who were either producers and/or frequent consumers of the beverage, were recruited to form the selection panel. For selection, the tests recommended by Associação Brasileira de Normas Técnicas [ABNT] (2016) were used, with adaptations of the tests used by Dutcosky (2013) tests of identification of stimuli of tastes and sensations and test of levels of intensity of a stimulus were used. For the beer off-flavors identification test, the off-flavors sensory kit by Brau Flavors® was used, which is purchased in 5 mL bottles that must be diluted in 1 L of beer. Those who obtained 100% accuracy in the identification test of stimuli of basic tastes and sensations, 100 % accuracy in the discriminative test for detection of stimulus intensity and at least 50 % accuracy in the identification test of off flavors of beers were selected.

For the survey of attributes, the network method described by Dutcosky (2013) was followed and to increase the consensus among judges, the analysis sheet was composed together and using the same terms for all judges, following the procedure performed by Gouton et al. (2021) for analysis of cookies in virtual reality.

After surveying the attributes and defining which terms would compose the analysis form, the Flash Profile technique was applied, which does not require prior training; therefore, no training was conducted. To carry out this method, 5 samples of beers were presented, namely: the standard beer, the beer produced with the yeast *S. boulardii* and three commercial beers of the styles Blond Ale, American Pale Ale and Weiss, taking into account the use of top-fermenting yeasts. The judges were asked to order the 5 samples according to the intensity of the evaluated attributes, with 5 being the sample with the highest intensity, 3 the intermediate sample and 1 the sample with the lowest intensity. The Flash or Free Profile descriptive sensory analysis is based on the principle that each individual describes different terms or descriptors for a sample, but the perception and sensation are the same.

The research was certified by the Ethics Committee in Research involving human beings at the Federal Technological University of Paraná (ECR/UTFPR – *Universidade Tecnológica Federal do Paraná*, from Portuguese), with Certificate of Presentation for Ethical Appreciation (CPEA) number 50428121.6.0000.0177. Participation was only possible by signing the Free and Informed Consent Term (FICT).

Statistical analysis

Statistical analysis for physical-chemical and bioactive compounds analysis was performed using the SAS system computer program for Windows version 9.4. Data were submitted to analysis of variance ($p \leq 0.05$). In case of statistical significance, the effects of the yeasts used were compared by the t-test ($p \leq 0.05$).

Sensory analysis was treated statistically through Generalized Procrustes Analysis using XLSTAT statistical software version 2020.5.

RESULTS AND DISCUSSION

Physicochemical results

The results obtained in the physicochemical analysis of the beers produced are shown in Table 1.

Table 1
Results of physicochemical analysis performed on the beers produced

Parameters	Saccharomyces cerevisiae	Saccharomyces cerevisiae var. boulardii	Literature	Reference
Alcohol % v/v (ABV)	4,59*	3,76	4,2 a 5,6	BJCP, 2021
Real Extract (°P)	4,19*	5,59	...	
Apparent Extract (°P)	2,46*	4,11	2,43	H. G. Silva et al., 2021
Original Extract (°P)	11,14*	11,17	> 5%	Ferreira, 2019
pH	4,36*	4,45	< 4,5 %	Jenkins et al., 2009
Color (EBC)	12ns	12	7	Ferreira, 2019
Bitterness (IBU)	15*	12	8 a 20	BJCP, 2021
Turbidity (EBC)	23,4*	46,8	6,6 a 25,34	H. G. Silva et al., 2021

* and ns represent, respectively, significant and non-significant by the t-test (p≤0.05) among the yeasts used to make the beers.

According to the data presented in Table 1, there was a significant difference in the attributes between the beers produced, except for color, which presented the same value for both treatments.

For this style of American beer, according to the Beer Judge Certification Program [BJCP] (2021) style guide, the alcohol percentage ABV must be between 4.2 and 5.6. The standard beer kept what was expected for the style, while the beer produced with the non-conventional yeast had an alcohol content below the minimum. Due to the high values of real extract and primitive extract in Formulation B, which was beer fermented with *S. boulardii*, low alcohol content was expected, as there was less sugar consumption during the fermentation process. The data corroborate with the evaluation made by Mendonça (2021), which found low values of alcohol content, between 3.08 and 3.44, in beers fermented with *S. boulardii* of the Pilsen, Indian Pale Ale and Belgian Golden Strong Ale styles, respectively. H. G. Silva et al. (2021) evaluated the influence of yeast immobilization on the physical-chemical quality and bioprocess variables and found values of 4.93% (ABV) for beer produced with free cells of *S. cerevisiae* US- 05. In beer fermented with mixed starters of conventional yeast and *S. boulardii*, Capece et al. (2018) found alcohol content values between 3.04 and 5.72% (ABV). However, in beer fermented only with *S. boulardii*, the content found was 3.93% (ABV), very close to that observed in the present study.

The real extract is related to all the solids present in the beer after fermentation, such as sugars and proteins, correcting with the alcohol content, sensorially the higher the real extract, the more full-bodied the beer will be. Apparent extract is related to the amount of extract or solids during fermentation, without correction for alcohol content. The primitive extract, by legislation, is the amount of extract in the wort 'which gave rise to beer' and this value must be greater than 5.0% by weight. These parameters are important to evaluate substrate availability for yeast and ethanol production, considering that these extracts are mainly formed by sugars (De Paula, 2019; Mendonça, 2021; Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2019).

For a Cream Ale beer using free cells of *S. cerevisiae*, Silva and Faria (2008) found data of 2.43 ºP of apparent extract, lower than the 2.46 and 4.11 found in the present work. Beer fermented with *S. boulardii* showed a significant difference compared to the standard formulation, this higher value may indicate that the yeast consumes less sugar and also explains the low alcohol content.

pH is an important microbiological control tool in beer, high pH values are not desirable. In the present study, pH data showed a significant difference between treatments but remained below 4.5, where bacteriostatic control is still efficient. The difference between the treatments may be related to the metabolism of the yeast *S. boulardii* during the fermentation process, the consumption of substrate is reduced, and consequently, the formation and excretion of organic acids are reduced (Mendonça, 2021).

There was no significant difference in color between treatments, possibly because it is a parameter linked mainly to the malt used and roasting. Ferreira (2019) described a color of 7.0 EBC for a Cream Ale style beer brewed only with

Pilsen malt, unlike this work where Castle Malting Chateau Munich and Castle Malting Chatea Cara Gold malts were added, which have a higher degree of roasting, where the color measured was 12 EBC.

According to the BJCP (2021), the bitterness in Cream Ale beers should be between 8 and 20 IBU, both beers produced in this study are within this range. As the same hops were used in both formulations and in equal concentrations, the significant difference in beer bitterness may be related to the isomerization of iso-α-acids during fermentation (Silva & Faria, 2008).

H. G. Silva et al. (2021) found values between 6.6 and 25.34 EBC of turbidity for Cream Ale beer fermented with *S. cerevisiae* with free and immobilized cells, being close to the value found for the standard beer of the present study. Formulation B showed turbidity 2 times greater than the standard formulation, which may be related to the attenuation of unconventional yeast and the presence of yeast in suspension, which can be evidenced by the subtle yeast aroma detected in the sensory analysis of the drink. Loser (2017) also identified a difference in yeast flocculation and sedimentation of 65 to 70% when compared to beers produced with conventional yeast.

Bioactive compounds

The quantification of bioactive compounds in the beers produced is presented in Table 2, in this study there was no characterization of these compounds.

Table 2
Results of the analysis of bioactive compounds carried out in the produced beers

Parameters	Saccharomyces cerevisiae	Saccharomyces cerevisiae var. boulardii	Literature	Reference
Total phenolic compounds (mg EAG. L-1)	861,5 ± 0,01ns	915,7 ± 0,06	272,7 a 304,1	Nunes Filho et al., 2021
Total flavonoid compounds (mg EQC. L-1)	3,5 ± 0,00*	6,4 ± 0,00	...	
Antioxidant activity - DPPH method (mmol TE. L-1)	66,5 ± 0,01*	84,6 ± 0,01	0,8 e 0,58	Nunes Filho et al., 2021
Antioxidant activity - FRAP method (mmol Fe2+. L-1)	2405,8 ± 0,03*	2547,5 ± 0,04	342,64 e 423,00	Nunes Filho et al., 2021
Antioxidant activity – ABTS method (mmol TE. L-1)	921,8 ± 0,03ns	899,8 ± 0,02	1850,83 a 2489,58	Vieira et al., 2021

* and ^{ns} represent, respectively, significant and non-significant by the t-test (p≤0.05) among the yeasts used to make the beers.

Within the wide variety of phenolic compounds, the most studied for beer are phenolic acids. Gallic acid is a natural phenolic acid and some of its biological effects may be associated with anticancer activity (Hanck-Silva, 2021).

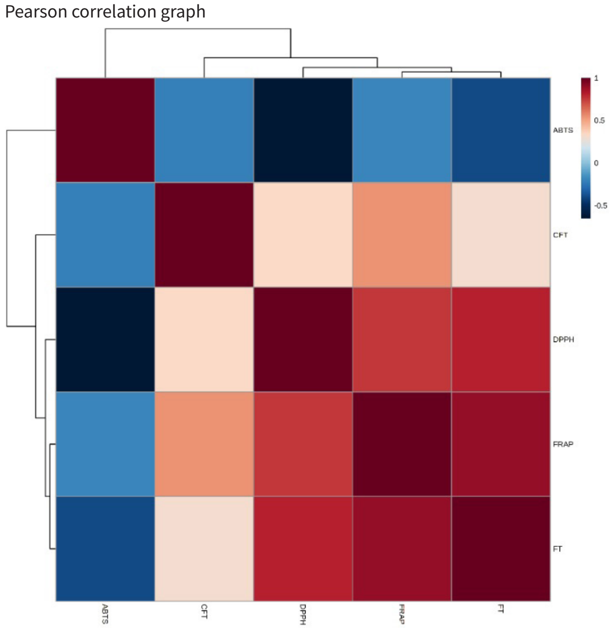
The values obtained for total phenolic compounds (TFC) in this study showed no significant difference between the yeasts used. In both beers the results found were superior to those obtained in Red Ale beers produced with yeast *S. cerevisiae* M07 and the addition of spices with antioxidant compounds, where the contents ranged from 272.7 to 304.1 mg EAG. L^{-1} (Nunes Filho et al., 2021). Viana et al. (2021) in their work quantified TFC in American Pale Ale style beer, using different strains of *S. cerevisiae*, where 18 compounds were quantified, among them the main phenolic acid found was chlorogenic acid in concentrations between 1.08 to 2.21 mg.L^{-1}. No data were found in the literature on the quantification of bioactive compounds for the specific Cream Ale style, but according to Nunes Filho et al. (2021) and R. N. P. Silva et al. (2021), ale-type beers have higher TFC levels due to the use of malts with higher roasting and greater beer color.

The total flavonoids (TF) contents were significantly higher in beer produced with non-conventional yeast *S. boulardii* compared to beer produced with conventional yeast. Capece et al. (2018) found an increase in antioxidant activity and polyphenol content when fermenting beer with mixed starters with *S. boulardii* yeast, compared to fermentations with single strains. Farinazzo et al. (2020) studied the influence of yeast *S. boulardii* on the fermentation kinetics of apple pulp and the antioxidant effect of quercetin and concluded that the yeast itself is more resistant to oxidizing agents and, together with quercetin, can increase bioavailability and antioxidant activity of apple pulp.

The study of the antioxidant activity of beers was carried out by the DPPH methods, which evaluates the power of reducing free radicals by the DPPH radical (2,2-diphenyl-1-picryl-hydrazyl); by the ABTS (2,2' azinobis [3-ethylbenzothiazoline-6-sulfonic acid]) radical scavenging method and by the FRAP ferric ion reduction method. In the DPPH and FRAP methods, it was possible to detect the significant influence of the yeast on the antioxidant activity of the beers. In the ABTS method, however, it was not possible to detect a significant difference in the antioxidant activity of the beers.

From the Pearson correlation graph (Fig.1) it is possible to observe a strong correlation between the flavonoid contents and the antioxidant activities by the DPPH and FRAP methods ($r > 0.85$), which may indicate that the antioxidant activity of beer is linked to mainly to the content of flavonoids and not to other phenolic compounds.

Figure 1
Pearson correlation graph



Nunes Filho et al. (2021) found values between 0.8 and 0.58 mmol TE.L^{-1} of antioxidant activity by the DPPH method, and values between 342.64 and 423.00 mmol TE.L^{-1} by the FRAP method in Red Ale beer with conventional yeast, values lower than those found in the present study. The antioxidant activity of fermented beers using mixed starters with *S. boulardii* showed DPPH values between 0.42 and 4.12 mmol TE.L^{-1}, which shows that the use of *S. boulardii* as the only strain increases up to 6x the redox power.

Vieira et al. (2021) in their study on the antioxidant activity of wheat beers found values ranging from 1850.83 to 2489.58 mmol TE.L^{-1} for the ABTS method that exceeds those obtained in this study. These values indicate the great influence of the use of wheat on the antioxidant activity of beers.

Beer fermented with *Saccharomyces boulardii,* a probiotic yeast derived from *S. cerevisiae*, offers notable functional health benefits due to its ability to enhance and preserve bioactive compounds such as phenolics and flavonoids. These compounds exhibit strong antioxidants and anti-inflammatory properties, contributing to the reduction of oxidative stress, protection against cardiovascular and neurodegenerative diseases, and modulation of the immune system (Capece et al., 2018; Valiente et al., 2023). In addition, *S. boulardii* supports gut health by surviving gastrointestinal passage and inhibiting pathogens, while phenolic compounds further promote a balanced microbiota, enhancing intestinal and systemic health (Lazo-Vélez et al., 2018). Flavonoids present in beer may also exert neuroprotective effects and positively influence mood via the gut–brain axis, particularly when combined with the probiotic action of *S. boulardii* (Paula et al., 2021). From a technological standpoint, phenolics improve beer's sensory attributes—such as flavor complexity, color stability, and foam retention—while also extending shelf life by reducing oxidation (Manshin et al., 2022). These combined effects position *S. boulardii*-fermented beer as a novel functional alcoholic beverage with promising health-promoting properties when consumed in moderation.

Sensory analysis

The team consisted of 19 judges, 12 men and 7 women aged between 21 and 44 years.

The number of attributes surveyed by the network method that made up the analysis form was 13. For appearance, the attributes of foam formation, foam persistence, color and turbidity were used. For aroma, the terms hop aroma and yeast aroma were used. For the flavor, the attributes were acid, bitter, sweet, refreshing, beer aftertaste and carbonation. For the texture, the density or full-bodied attribute was evaluated.

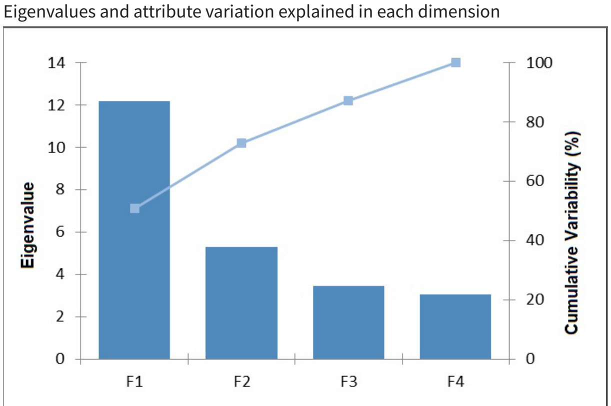
Araújo (2019) used the Check-All-That-Apply method for sensory characterization and raised 20 main descriptors for the drink, including citrus, acid, sweet and refreshing. Araújo et al. (2003) also used the descriptive terms color, yeast aroma, sweet taste, bitter taste and carbonation. Therefore, the evaluated attributes are commonly used for the sensory description of beer, in accordance with the literature.

The variety of terms identified in the sensory analysis, although rich and capable of explaining the differences between the beverages, makes direct interpretation of the results more difficult. Therefore, factor analysis is applied to synthesize the data by grouping attributes that exhibit similar behavior (Moreira, 2022).

Fig. 2 shows the distribution of eigenvalues and the accumulated explained variance, resulting from the factor analysis applied to the sensory attributes of the beer. On the horizontal axis are the dimensions extracted from the analysis, labeled F1, F2, F3, and F4, corresponding to the first four principal factors. The left vertical axis represents the individual eigenvalues, indicating the amount of variance explained by each factor separately. The right vertical axis displays the cumulative variance, in percentage terms, showing that the first two factors (F1 and F2) together explain more than 70% of the total data variance.

This result supports the use of a two-dimensional solution to represent the relationships among the evaluated sensory atributes (Dutcoski, 2013).

Figure 2
Eigenvalues and attribute variation explained in each dimension



Note: F1 to F4 represent the factors extracted from the analysis, each grouping related sensory attributes. The bars show the eigenvalues (left axis), while the line shows the cumulative variability explained (right axis). The first two dimensions together explain over 70% of the total variance, supporting the use of a two-dimensional representation.

The interpretation of the structure presented in Fig. 2 is supported by the data in Table 3. In dimension 1, the highest positive and negative correlations observed were foam formation, foam persistence, color, hop aroma, yeast aroma, acid taste, bitter taste, sweet taste, refreshing and carbonation. In dimension 2, the most intense positive and negative correlations were turbidity, beer aftertaste and full-bodied texture (Table 3 and Fig. 3).

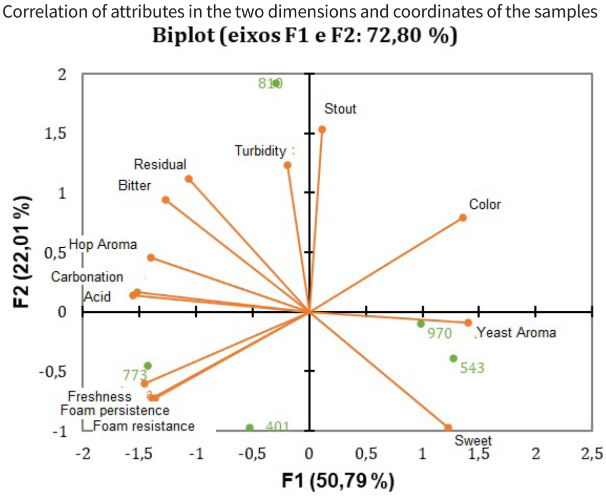
Table 3
Correlations between the dimensions of the initial consensus configuration and the attributes

Parameters	Dimension 1	Dimension 2
Foam formation	-0,878	-0,459
Foam persistence	-0,863	-0,458
Color	0,858	0,498
Turbidity	-0,119	0,778
Hop aroma	-0,884	0,290
Yeast aroma	0,888	-0,059
Acid taste	-0,983	0,090
Bitter taste	-0,801	0,597
Sweet taste	0,776	-0,613
Refreshing	-0,922	-0,381
Beer aftertaste	-0,675	0,705
Carbonation	-0,959	0,105
Full-bodied texture	0,070	0,970

Fig. 3 presents the correlation of attributes in the two dimensions and the discrimination of samples within these two dimensions. The attributes that best described the standard beer and the beer produced with *S. boulardii* yeast were sweetness, yeast aroma and color, with both beers differing from the commercial beers used in the analysis, as also shown in the dendrogram in Fig. 4. The attributes that best described the commercial Blond Ale style beer were turbidity, aftertaste, bitterness and beer body. For the American Pale Ale and Weiss style commercial beers, the attributes that best described were foam formation, foam persistence and refreshing. The attributes: hop aroma, acidity and carbonation did not reach a consensus among judges but were better evaluated in commercial samples.

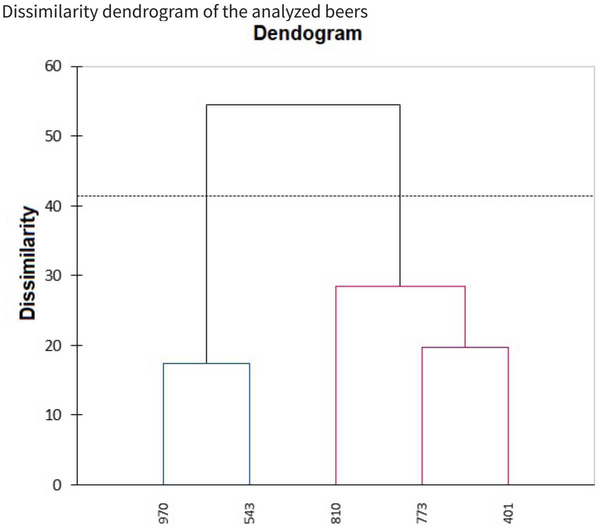
The yeast aroma presents in the beers produced was subtle but represents a possible failure in the production process. It cannot be indicative of differences from non-conventional yeast, as the standard yeast had the same aroma. Yeast aroma and carbonation vectors form an angle close to 180°C to each other (Fig.3.), showing a possible negative linear correlation, for this reason, the low carbonation of the produced beers has a great influence on the perception of yeast aroma.

Figure3
Correlation of attributes in the two dimensions and coordinates of the samples



Note: Code 970- standard formulation A, Code 543- formulation B with S. boulardii. Code 810- Blond Ale style commercial beer. Code 773- American Pale Ale style commercial beer. Code 401- Weiss style commercial beer.

Figure 4
Dissimilarity dendrogram of the analyzed beers



Note: Code 970- standard formulation A, Code 543- formulation B with S. boulardii. Code 810- Blond Ale style commercial beer. Code 773- American Pale Ale style commercial beer. Code 401- Weiss style commercial beer.

According to the BJCP (2021), the Cream Ale style has a subtle aroma without a predominance of malt or hops, the color varies from straw yellow to moderate gold, the foam formation is low to medium, and the carbonation is medium to high with reasonable retention or persistence. For flavor, low hop bitterness and low to moderate maltiness and sweetness, neither malt nor hops dominate the palate. Historically it is an ale version to compete with the lagers that were produced a lot in Canada and the Northern States. In short, it is a light and refreshing beer. Accordingly, the Generalized Procrustes Analysis showed that the use of *S. boulardii* as a fermenting agent did not lead to major differences in the sensory perception of the drink, as the standard beer had similar attributes.

Beers fermented with *Saccharomyces boulardii* have a distinctly cleaner and more balanced sensory profile compared to those produced with traditional *S. cerevisiae* strains, characterized by a neutral aroma, subtle notes of fresh yeast and bread, lower production of fruity esters and volatile phenols, and a pleasantly light acidity (Capece et al., 2018; Valiente et al., 2023). The flavor tends to be mild, with moderate bitterness and slight residual sweetness, while the body is light to medium, with a good mouthfeel and well-integrated carbonation. Visually, moderate turbidity is related to the presence of viable *S. boulardii* cells, which is important for probiotic functionality, in addition to contributing to head and color stability due to greater retention of phenolic compounds (Lazo-Vélez et al., 2018; Paula et al., 2021). Sensory tests have demonstrated good public acceptance, especially in lighter styles such as Witbier, Session Ales and fruit beers, with aroma and flavor stability maintained for up to 30 days of storage (Capece et al., 2018).

CONCLUSION

This work aimed to produce Cream Ale style beer using *Saccharomyces cerevisiae* var. *boulardii* as fermenting agent, to evaluate a possible substitute for conventional yeast for the brewing industry.

The Cream Ale style beer produced with *Saccharomyces cerevisiae* var. *boulardii* showed significant differences related to the attenuation of the wort, as well as the alcohol content and the real extract, so the yeast needs a longer fermentation and maturation time to reach the production standards achieved with the yeast *S. cerevisiae*. Regarding bioactive compounds, beer made with *S. boulardii* showed values higher than those found in the literature, indicating the strong appeal of healthiness with the consumption of the drink and the possible beneficial effects of its ingestion. The only data found superior were related to beer produced with wheat.

Beer fermented with *S. boulardii* did not show significant sensory differences in relation to beer produced with conventional *Saccharomyces cerevisiae*, but both showed great differences when compared to commercial beers. In this study, the beer was produced in an artisanal way, which may indicate possible improvements in this process.

Future developments involving the fermenting agent *S. boulardii* encompass the study of viable cells during the shelf life of the beverage and also include an economic analysis of production with this yeast, considering possibilities for replication. Other analyses also include the replication of the present study in different beer styles.

REFERENCES

Ansari, F., Alian Samakkhah, S., Bahadori, A., Jafari, S. M., Ziaee, M., Khodayari, M. T., & Pourjafar, H. (2023). Health-promoting properties of Saccharomyces cerevisiae var. boulardii as a probiotic; characteristics, isolation, and applications in dairy products. *Critical Reviews in Food Science and Nutrition, 63*(4), 457-485. https://doi.org/10.1080/10408398.2021.194957

Araújo, F. B., Silva, P. H. A., & Minim, V. P. R. (2003). Perfil sensorial e composição físico-química de cervejas provenientes de dois segmentos do mercado brasileiro. *Food Science and Technology*, *23*(2), 121-128. https://doi.org/10.1590/S0101-20612003000200004

Araújo, F. R. (2019). *Impacto sensorial da microbiota no off-flavour da cerveja artesanal session ipa durante o armazenamento*. [Master's Thesis, Federal University of Ceará]. UFC Repository http://www.repositorio.ufc.br/handle/riufc/50772

Associação Brasileira de Normas Técnicas. (2016). *Análise sensorial – guia para a seleção, treinamento e monitoramento de avaliadores selecionados e de especialistas ou experts (Norma ABNT NBR ISO 8586:2024)*. https://abntcatalogo.com.br/pnm.aspx?Q=TmVPTVhyTS9qUEp3NDFuU1E5cjY2bnZkUkpTcFg1UUsrd1BIRkVZb3ozdz0=

Beer Judge Certification Program. (2021). *Beer style guidelines*. https://www.bjcp.org/style/2021/beer/

Bortoleto, G. G., & Gomes, W. P. C. (2020). Determination of volatile organic compounds in craft beers by gas chromatography and headspace sampling. *Research, Society and Development*, *9*(9), Article e600997746. https://doi.org/10.33448/rsd-v9i9.7746

Capece, A., Romaniello, R., Pietrafesa, A., Siesto, G., Pietrafesa, R., Zambuto, M., & Romano, P. (2018). Use of *Saccharomyces cerevisiae* var. *boulardii* in co-fermentations with *S. cerevisiae* for the production of craft beers with potential healthy value-added. *Internacional Journal of Food Microbiology*, *284,* 22-30. https://doi.org/10.1016/j.ijfoodmicro.2018.06.028

De Paula, B. R. (2019). *Evaluation of technological and functional potential of saccharomyces boulardii for probiotic beers development.* [Doctoral Thesis, Federal University of Rio de Janeiro]. UFRJ Repository https://ppgcal.iq.ufrj.br/wp-content/uploads/Tese_Breno-Pereira-de-Paula.pdf

Dutcosky, S. D. (2013). *Análise sensorial de alimentos* (4th ed.). Champagnat.

European Brewery Convention. (2020). *9.8—Bitterness of Beer; European Brewery Convention*. Hans Carl Getränke-Fachverlag: Nürnberg, Germany.

Farinazzo, F. S., Madeira, T. B., Fernandes, M. T. C., Mauro, C. S. I., Tomal, A. A. B., Nixdorf, S. L., & Garcia, S. (2020). Organic and conventional apple fermented by Saccharomyces boulardii – The effect of the antioxidant quercetin on cellular oxidative stress. *Bristish Food Journal, 123*(2), 520-534. https://doi.org/10.1108/BFJ-07-2019-0564

Ferreira, L. P. (2019). *Análise das cervejas tipo cream ale, pilsen e saison, segundo as especificações legais.* [Undergraduate Thesis, Federal University of Paraíba]. UFPB Repository. https://repositorio.ufpb.br/jspui/handle/123456789/15563

Galli, V., Messias, R. S., Perin, E. C., Borowski, J. M., Bamberg, A. L., & Rombaldi, C. V. (2016). Mild sat stress improves strawberry fruit quality. *LWT, 73*, 693-699. https://doi.org/10.1016/j.lwt.2016.07.001

Gouton, M., Dacremont, C., Trystam, G., & Blumenthal, D. (2021). Validation of food visual attribute perception in virtual reality. *Food Quality and Preference, 87*, Article e104016. https://doi.org/10.1016/j.foodqual.2020.104016

Hanck-Silva, G. (2021). *Avaliação do potencial do ácido gálico encapsulado em metal-organic frameworks funcionalizados com cetuximabe no tratamento do cancer de próstata*. [Doctoral Thesis, São Paulo State University]. UNESP Repository. https://repositorio.unesp.br/handle/11449/215888

Instituto Adolfo Lutz. (2008). *Métodos químicos e físicos para análise de alimentos* (1st ed.). IMESP.

Issock, P. B., Mpinganjira, M., & Roberts-Lombard, M. (2023). Beyond sustainable consumption practices: Linking organic food consumption to hedonic and eudaimonic well-being. *Appetite, 188,* Article e106633. https://doi.org/10.1016/j.appet.2023.106633

Jenkins, C. L., Lawrence, S. J., Kennedy, A. I., Thurston, P., Hodgson, J. A., & Smart, K. A. (2009). Incidence and Formation of Petite Mutants in Lager Brewing Yeast *Saccharomyces Cerevisiae* (Syn. *S. Pastorianus*) Populations. *Journal of the American Society of Brewing Chemists*, *67*(2), 72-80. https://doi.org/10.1094/ASBCJ-2009-0212-01

Kirin Beer University Report. (2020). *Global beer consumption by country in 2019*. https://www.kirinholdings.co.jp/english/news/2020/1229_01.pdf

Lazo-Vélez, M. A., Serna-Saldívar, S. O., Rosales-Medina, M. F., Alvear-Tinoco, M., & García-Briones. (2018). Application of *Saccharomyces cerevisiae var. boulardii* in food processing: a review. *Journal of Applied Microbiology, 125*(4), 943-951. https://doi.org/10.1111/jam.14037

Loser, A. L. (2017). *Avaliação do potencial de Saccharomyces boulardii na produção de cerveja probiótica*. [Undergraduate Thesis, University Center - Catholic University of Santa Catarina]. Católica SC Repository. http://app.catolicasc.org.br/imagens_biblio/000091/0000918C.pdf

MacFarland, L. V., & Li, T. (2025). Efficacy and safety of *Sacchomyces boulardii* CNMC I-745 for the treatment of pediatric acute diarrhea in China: a systematic review and meta-analysis. *Frontiers in Cellular and Infection Microbiology, 15*, 1587792. http://doi.org/10.3389/fcimb.2025.1587792

Mafra, G. P. (2018). *Análise físico-química de cerveja american lager maturada com pimenta rosa (aroeira)*. [Undergraduate Thesis, Federal University of Rio Grande do Norte]. UFRN Repository. https://repositorio.ufrn.br/handle/123456789/38840

Manshin, I., Ramírez-Cota, J. M., & Borges, F. (2022). Probiotic yeast in beer fermentation: A review. *Beverages, 10*(4), 109. https://doi.org/10.3390/beverages10040109

Mendonça, A. (2021). *Emprego de Saccharomyces cerevisiae var. boulardii no processo de elaboração de cervejas com potencial probiótico*. [Undergraduate Thesis, State University of Rio Grande do Sul]. UERGS Repository. https://repositorio.uergs.edu.br/xmlui/handle/123456789/2030

Ministério da Agricultura, Pecuária e Abastecimento. (2019). Instrução Normativa n.º 65/2019. *Diário Oficial da União*, Seção 1, 239 (dezembro), 31. https://www.in.gov.br/web/dou/-/instrucao-normativa-n-65-de-10-de-dezembro-de-2019-232666262

Moreira, D. B. (2022). *Correlação entre características físicas e atributos sensoriais de Coffea arabica L. por meio de análise de fatores*. [Master's Thesis, Federal University of Viçosa]. UFV Repository. https://doi.org/10.47328/ufvcrp.2022.011

Moreira Terhaag, M., Sakai, O. A., Ruiz, F., Garcia, S., Bertusso, F. R., & Prudêncio, S. H. (2025). The Probiotication of a Lychee Beverage with *Saccharomyces boulardii*: An Alternative to Dairy-Based Probiotic Products. *Foods*, *14*(2), 156. https://doi.org/10.3390/foods14020156

Nunes Filho, R. C., Galvan, D., Effting, L., Terhaag, M. M., Yamashita, F., Benassi, M. T., & Spinosa, W. A. (2021). Effects of adding spices with antioxidants compounds in red ale style craft beer: A simplex-centroid mixture design approach. *Food Chemistry, 365*, Article e130478. https://doi.org/10.1016/j.foodchem.2021.130478

Park, Y. K., Ikegaki, M., Abreu, J. A. S., & Alcici, N. M. F. (1998). Estudo da preparação dos extratos de própolis e suas aplicações. *Food Science and Technology*, *18*(3), 313-318. https://doi.org/10.1590/S0101-20611998000300011

Paula, B. P., Lago, H. S., Firmino, L., & Lemos Jr, W. J. F. (2021). Growth optimization of *Saccharomyces boulardii* in synthetic wort for functional alcoholic beverages. *LWT - Food Science and Technology*, *135*, 110057. https://doi.org/10.3389/fmicb.2019.02092

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Ryabtseva, S. A, Khramtsov, A. G., Sazanova, S. N., Budkevich, R. O., Fedortsov, N. M., & Veziryan, A. A. (2023). The probiotic properties of Saccharomycetes (Review). *Applied Biochemistry and Microbiology, 59*(2), 111-121. https://doi.org/10.1134/S0003683823010088

Sgorla, A. F. (2024). Fermentations of authenticity: the construction of quality, taste, and price in the craft beer market in Brazil. *Cuadernos De Educación Y Desarrollo*, *16*(5), e4142. https://doi.org/10.55905/cuadv16n5-020

Silva, H. G., Souza, P. G. de, & Pinheiro, C. L. (2021). Estudo da reutilização de leveduras imobilizadas sobre a qualidade da cerveja Cream Ale/ Study on the reuse of immobilized yeasts on the quality of Cream Ale beer. *Brazilian Journal of Development*, *7*(4), 43083-43095. https://doi.org/10.34117/bjdv7n4-665

Silva, P. H. A., & Faria, F. C. (2008). Avaliação da intensidade de amargor e do seu princípio ativo em cervejas de diferentes características e marcas comerciais. *Food Science and Technology*, *28*(4), 902-906. https://doi.org/10.1590/S0101-20612008000400021

Silva, R. N. P., Dias, J. F., & Koblitz, M. G. B. (2021). Beers: relationship between styles; phenolic compounds and antioxidant capacity. *Research, Society and Development*, *10*(3), Article e42210313471. https://doi.org/10.33448/rsd-v10i3.13471

Singh, R., & Rathore, D. (2018). Oxidative stress defence responses of wheat (*Triticum aestivum* L.) and chilli *Capsicum annum* L.) cultivars grown under textile effluent fertilization. *Plant Physiology and Biochemistry, 123,* 342-358. http://dx.doi.org/10.1016/j.plaphy.2017.12.027

Souza, H. F. de, Carosia, M. F., Pinheiro, C., Carvalho, M. V. de, Oliveira, C. A. F. de, & Kamimura, E. S. (2022). On probiotic yeasts in food development: *Saccharomyces boulardii*, a trend. *Food Science and Technolology*, *42*, e92321. https://doi.org/10.1590/fst.92321

Toledo, A. L., Silveira, P. M., & Capuci, A. P. S. (2018, november 30 to december 01). Produção de Cerveja Artesanal [Paper presentation]. *II Encontro de Desenvolvimento de Processos Agroindustriais – EDEPA*, Uberaba, Minas Gerais, Brazil. http://dspace.uniube.br:8080/jspui/handle/123456789/902

Tozzeto, L. M. (2017). *Produção e caracterização de cerveja artesanal adicionada de gengibre (Zingiber officinale)*. [Master's Thesis, Federal Technological University of Paraná]. UTFPR Repository. http://repositorio.utfpr.edu.br/jspui/handle/1/2451

Valiente, S., Díaz, A. B., Durán-Guerrero, E., & Lasanta, C. (2023). Development and characterization of probiotic beers with *Saccharomyces boulardii* as an alternative to brewer's yeast. *Food Research International*, *174*, 113170. https://doi.org/10.1016/j.foodres.2023.113170

Viana, A. C., Pimentel, T. C., Vale, R. B., Clementino, L. S., Ferreira, E. T. J., Magnani, M., & Lima, M. S. (2021). American Pale Ale craft beer: Influence of brewer's yeast strains on the chemical composition and antioxidant capacity. *LWT, 152,* Article 112317. https://doi.org/10.1016/j.lwt.2021.112317

Vieira, A. C. G., Lacerda, E. C. Q., Sant'Ana, G. C. F., Leão, M. H. M. R., & Amaral, P. F. F. (2021). Antioxidant activity in commercial wheat beer. *Brazilian Journal of Development, 7*(2), 15217-15222. https://doi.org/10.34117/bjdv7n2-239