

Production of functional beer with the addition of probiotic: *Saccharomyces boulardii*

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Received: 01/20/2021 | Reviewed: 01/23/2021 | Accept: 01/26/2021 | Published: 02/03/2021

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Abstract

Industries have been investing in the development of functional products, such as the ones produced with probiotic microorganisms. The aim of this study was to develop a Pilsen-style beer with functional properties through the addition of the *Saccharomyces boulardii* probiotic. The beer was fermented with low-fermentation commercial yeast. The probiotic culture was added next, evaluating its probiotic potential. Microbiological, sensorial and physicochemical aspects were evaluated in the obtained products. The *S. boulardii* strain presented probiotic viability after the confirmation of the resistance to acid, bile salts and ethyl alcohol tests, maintaining superior levels of colony-forming to the indicated by the legislation during the 28 days storage. The beer with probiotic addition has not differed sensorially from the commercial beer concerning the aroma, flavor, general appearance and color. The results of sensorial, physicochemical and microbiological analysis showed the viability of the production of probiotic beer with the addition of *S. boulardii*.

Keywords: Yeasts; Probiotics; Sensory properties.

Resumo

As indústrias têm investido no desenvolvimento de produtos funcionais, como os produzidos com microrganismos probióticos. O objetivo deste estudo foi desenvolver uma cerveja do tipo Pilsen com propriedades funcionais por meio da adição do probiótico *Saccharomyces boulardii*. A cerveja foi fermentada com fermento comercial de baixa fermentação. A seguir, a cultura probiótica foi adicionada, avaliando seu potencial probiótico. Os aspectos microbiológicos, sensoriais e físico-químicos foram avaliados nos produtos obtidos. A cepa de *S. boulardii* apresentou viabilidade probiótica após a confirmação da resistência aos testes de ácido, sais biliares e álcool etílico, mantendo níveis de formação de colônias superiores aos indicados pela legislação durante os 28 dias de armazenamento. A cerveja com adição de probióticos não diferiu sensorialmente da cerveja comercial quanto ao aroma, sabor, aspecto geral e cor. Os resultados das análises sensoriais, físico-químicas e microbiológicas mostraram a viabilidade da produção de cerveja probiótica com a adição de *S. boulardii*.

Palavras-chave: Leveduras; Probióticos; Propriedades sensoriais.

Resumen

Las industrias han estado invirtiendo en el desarrollo de productos funcionales, como los producidos con microorganismos probióticos. El objetivo de este estudio fue desarrollar una cerveza estilo Pilsen con propiedades funcionales mediante la adición del probiótico *Saccharomyces boulardii*. La cerveza se fermentó con levadura comercial de baja fermentación. A continuación, se agregó el cultivo probiótico, evaluando su potencial probiótico. Se evaluaron aspectos microbiológicos, sensoriales y fisicoquímicos en los productos obtenidos. La cepa *S. boulardii* presentó viabilidad probiótica tras la confirmación de las pruebas de resistencia al ácido, sales biliares y alcohol etílico, manteniendo niveles de formación de colonias superiores a los indicados por la legislación durante los 28 días de almacenamiento. La cerveza con adición de probióticos no se ha diferenciado sensorialmente de la cerveza comercial en cuanto a aroma, sabor, apariencia general y color. Los resultados del análisis sensorial, fisicoquímico y microbiológico mostraron la viabilidad de la producción de cerveza probiótica con la adición de *S. boulardii*.

Palabras clave: Levaduras; Probióticos; Propiedades sensoriales.

1. Introduction

Evidences suggest that the awareness of the consumer about health and well-being has increased greatly in recent times. In addition to that, it is known that food has a large potential in reducing the risk of diseases, which has caused an increase in the offer of functional foods in the market (Chrysochou, 2014; Krebs *et al.*, 2018).

The intestinal microbiota affects several aspects related to human health (Ventura *et al.*, 2018), performing an important role as modulators of metabolic responses and the immunological system (Duda-Chodak *et al.*, 2015; Ventura *et al.*, 2018). Probiotics have the capacity of altering the composition of the intestinal microbiota and preventing the growth of pathogenic bacteria (Almada *et al.*, 2015). Therefore, probiotics are defined as organisms which, when administered in proper quantities, benefit the host's health (FAO/WHO, 2002).

The *Saccharomyces boulardii* is a very commonly yeast used as probiotic. This yeast can be commercialized as a dietetic supplement and it also presents several properties which confirm its effect as a probiotic agent, such as tolerance to the stomach acids and bile salts, besides the capacity of surviving body temperature (37 °C) and the intestinal tract's environment (McFarland, 2010; Kelesidis & Pothoulakis, 2012). *S. boulardii* is used as a preventive and therapeutic source for the cure for diarrhea (McFarland, 2010; Rajkowska *et al.*, 2012).

Beer is a popular beverage, widely consumed all over the world and, even not being so common, it can be an interesting food source or the providing of probiotics (Sohrabvandi *et al.*, 2010). The beer industry has been developing new technologies for the production of beers which promote benefits to health such as functional beers, which are produced through the use of non-conventional ingredients that are able to produce or transform some production input into compounds which are beneficial to health (Basso *et al.*, 2016).

Concerning this concept, this study aimed at developing a functional beer, with the addition of *S. boulardii*, assessing the cultivation of this strain as well as its probiotic potential. Besides that, we also verified the standard quality of the beer produced and its sensorial acceptance.

2. Methodology

2.1 Cultivation of *Saccharomyces boulardii* and the fermentative process monitoring

The preparation of *Saccharomyces boulardii* inoculum was realized according to Muller *et al.* (2007). The culture was obtained from the commercialized medicine Floratil® (Merck), in which each 100 mg capsule contains about 2×10^8 cells of *Saccharomyces boulardii* in freeze-dried form. The pre-inoculum was prepared in aseptic conditions, using 100 mg of *S. boulardii*, which was transferred to 100 mL of sterilized culture media containing: anhydrous glucose (10.0 g L^{-1}), meat peptone (2.0 g L^{-1}), yeast extract (2.0 g L^{-1}), phosphate monopotassium (0.60 g L^{-1}), urea (0.36 g L^{-1}), ammonium sulfate (0.12 g L^{-1}), magnesium sulfate (0.24 g L^{-1}) and water enough to complete 1 L. After 12 h, the optic density of the inoculum was measured to provide the cell concentration, adopting a minimum limit of 3.9 g L^{-1} . For the cultivation, 12 Erlenmeyer bottles were prepared with 50 mL of solution (45 mL of sterilized culture media and 5 mL of pre-inoculum) in a biologic-secure cabin. The fermentative process was monitored at initial time (t_0) and in 1 h intervals until the total time of 12 h.

In order to determine the cell concentration, the method described by Schmidell *et al.* (2001) was used. The culture media (10 mL) were filtered in cellulose membranes of $0.80 \text{ }\mu\text{m}$ and dissected at $40 \text{ }^\circ\text{C}$ until a constant weight. The cell concentration was calculated by the relation between the mass of each dissected membrane and the volume of the filtered sample. The absorbance was measured in a spectrophotometer at 600 nm, using the means of cultivation as white. A calibration curve was prepared with 0.01 to 0.60 g L^{-1} of cells ($R^2 = 0.99$).

The glucose consumption was quantified by the 3,5-dinitrosalicylic acid method, as described by Miller (1959), being the absorbance read in spectrophotometer at 540 nm. A standard curve prepared with glucose solutions - 0.1 to 1.0 g L⁻¹ - was used ($R^2 = 0.99$).

For the quantification of the ethanol produced during cultivation, the extracts obtained at different times were filtered in membranes of 0.80 µm (Sartorius) and the filtrate was analyzed by gas chromatography supplied with *headspace* injector. The chromatographic conditions were: oven temperature of 100 °C for 15 minutes, injector at 230 °C; detector at 250 °C; split: 1:10; dragging gas: hydrogen with flow rate of 1.0 mL min⁻¹. The obtained data were treated by the Microquímica software. The calibration curve was obtained through solutions of ethanol in the concentrations of 0.5 to 5.0% (w/w) ($R^2 = 0.99$) (Krebs *et al.*, 2018).

The pH in the conducted experiments was determined in incubator agitator through the immersion of a pH meter electrode, after its calibration with standard solutions of pH 4.01 and 7.01 (Horwitz & Latimer, 2005).

2.2 *S. boulardii* strain test of resistance to pH, bile e ethanol

For the tolerance tests, the cells of *S. boulardii* were reactivated and cultivated as described in item 2.1 and analyzed according to Muller *et al.* (2007). The assessment of resistance to bile (at concentrations of 0.15, 0.2 and 0.3% of bile salts) and pH (1.0, 2.0 and 3.0 adjusted with chloridric acid) was realized by the plating surface methodology in potato dextrose agar (PDA) and incubated for at 30 °C. The plating was conducted or cultivation times of 30, 60 and 90 minutes. In order to assess the resistance to ethanol, absolute ethanol was added at concentrations of 1, 2, 3, 4 e 5% (v/v). The samples were incubated in Shaker at 150 rpm and 30 °C. The cultivation was carried on for 24 h and after this period, the previously described plating was conducted.

2.3 Craft beer production

The activation of the *S. boulardii* probiotic culture was realized by mixing 1 g of dry cell in a glucose solution 10 g mL⁻¹ (previous autoclaved at 121 °C for 15 min). The sachet of yeast was sterilized with gaze dipped in alcohol 70% and inoculated in a biological secure chamber. After the inoculation, the sample was taken to the incubator with agitation of 150 rpm at 30 °C for 3 h (Muller *et al.*, 2007).

The wort used for the production of the probiotic beer was obtained at Heineken brewery Ponta Grossa (Paraná)'s unit. The wort used was the one from Pilsen beer, which was bottled in 600 mL capacity bottles, pasteurized at 10 UP (pasteurization unit) twice. Next, the wort was refrigerated (2 – 6 °C) until the moment of usage. The experiments in beer production were conducted in an 8-liter capacity stainless steel bioreactor, which was connected to a thermostatic bath for control of fermentation temperature. The culture of low-fermentation beer yeast *Saccharomyces cerevisiae* w 34/70 (Fermentis®) with a proportion of dry cell to worth of 1:1 (g L⁻¹) was added to the Pilsen-type wort. The worth was aired by mechanical agitation for incorporating oxygen. The fermentation occurred at a controlled temperature of 15 °C for primary fermentation. The measure of °Platô was checked every 8 h in order to verify its end. After the fermentation, the beer was bottled and pasteurized at 60 °C for 10 min (10 UP) (Rajkowska *et al.*, 2012, Ceccaroni *et al.*, 2018).

After the pasteurization, 5 to 10 mL of pre-activated probiotic culture were added to the beers which would be enriched with *S. boulardii*. Besides that, 7 g L⁻¹ of glucose were added in order to maintain the fermentation of the culture, ensuring the production of CO₂ in the final product (Rajkowska *et al.*, 2012). The beers were stored in a refrigerator at 4 °C.

2.4 Microbiological and physicochemical analysis of the produced beers

Microbiological and physicochemical analyses of the samples were conducted in the period of 7, 14 and 28 days. The number of viable *S. boulardii* cells was quantified by surface plating in PDA culture media (Rajkowska *et al.*, 2012).

The physicochemical analysis of the beers was carried according to AOAC (Horwitz & Latimer, 2005): ethanol content (984.14), diacetyl (978.11), volatile acidity (935.23), moisture (method 925.09), ashes (method 923.03), protein (method 920.87), bitterness (method 970.11), pH (method 945.10), color (method 976.08), total and volatile acidity (method 945.08). Superior alcohols and esters were quantified by direct injection into a gas-liquid chromatography system (GC-2014; Shimadzu) and turbidity was determined in Hach 2100AN (Nephelometer, Hach Co, Loveland).

The original extract, total dry extract and defatted dry extract, apparent extract and degree of fermentation were calculated by using the *Beer Calc* software, from the *Scandinavian School of Brewing* (SSB), using the values obtained in the other conducted physical-chemical analysis (SSB, 2016).

2.5 Sensorial Analysis

To sensorial analysis the recommendation of Ramsey *et al.* (2018) was followed. Thus, 50 untrained tasters were recruited from the Federal University of Santa Catarina (UFSC) with ages ranging from 18 to 30 years old. The consumers' affective methods consisted in hedonic tests using a structured 9-points hedonic scale ranging from 1 (extremely disliked) to 9 (extremely liked). The evaluated attributes were: global appearance, aroma, flavor, body, acidity and color. The tasters received three codified beer samples (~50 mL) at temperatures of 7 to 12 °C, which were presented randomly in plastic cups codified with 3 digits. Three samples of beer were used: (A) Pilsen beer with no addition of probiotic, (B) Pilsen beer with addition of probiotic and (C) commercial Pilsen beer. The approval of the Ethical Committee in Research with Human of the Federal University of Santa Catarina was granted before the beginning of this analysis.

2.6 Statistical Analysis

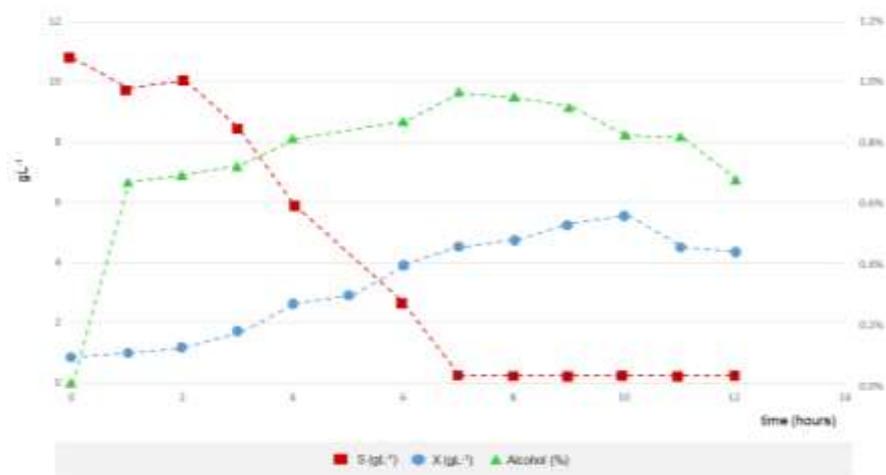
The analysis of Variance (ANOVA) was conducted in order to detect statistically relevant differences for the sensorial attributes between the beers. The Tukey test ($p < 0.05$) was conducted to compare the averages. The results were evaluated with the software EXCEL.

3. Results and Discussion

3.1 *S. boulardii* cultivation kinetics

Figure 1 presents the parameters evaluated in the *S. boulardii* cultivation kinetics with an initial concentration of 10 g L⁻¹ in the period of 12 h. The evaluated parameters were cellular concentration (X), glucose consumption (S) and production of ethanol concentration of 10 g L⁻¹ in the period of 12 h. It can be observed that *S. boulardii* presented a velocity of growth in exponential stage (2 to 4 h) in which the curve had an acute inclination due to favorable factors and conditions for the metabolism, where cellular growth is at maximum. After that, the growth becomes linear during the 12 h of the fermentative process, which indicates the presence of residual glucose. Another possible factor for the reducing of cellular growth is the production of ethanol, as observed by Sulieman *et al.* (2018) in the fermentation of *S. cerevisiae*.

Figure 1. Cellular concentration (X - $g L^{-1}$), concentration of consumed glucose (S - $g L^{-1}$) and alcohol production (% v/v) of *S. boulardii* cultivation

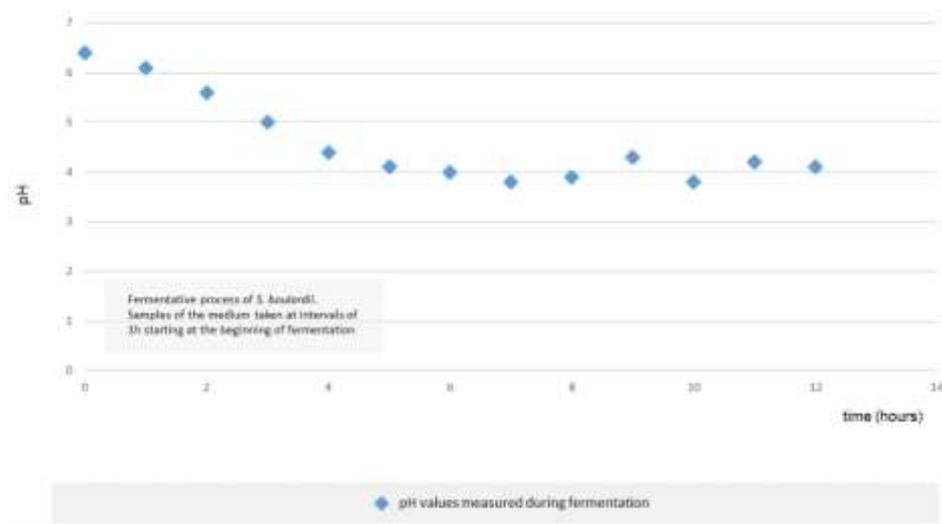


Source: Authors

In the early stages of the fermentation process, glucose was used essentially for cultivation. The consumption of the substrate was practically total in 12 h of fermentation of *S. boulardii*. Putra *et al.* (2013) observed the same for the fermentative process of *S. cerevisiae* in 28 h. The production of ethanol started dropping after 7 h of fermentation. The consumption of ethanol, glycerol and other organic acids happen due to metabolic oxidation by the cell during the fermentative process in order to generate more ATP and biomass, but only in aerobiosis (Schmidell *et al.*, 2001).

Figure 2 presents the results for the analysis of the pH during the fermentative process of the *S. boulardii*, which indicate a decrease in the values in the 12 h of fermentation. A study of the growth of *S. boulardii* has reported the same behavior for the parameter pH in 12 h of process (Muller *et al.*, 2007).

Figure 2. Results of the pH parameter of the experiments of *S. boulardii* cultivation



Source: Authors

Ribéreau-Gayon and Peynaud (1996) reported that the pH may possibly decrease due to the formation of organic acids (succinic, lactic, acetic and other acids) with a consequent increase of the acidity of the fermentation media.

3.2 Resistance of the *S. boulardii* to pH, bile salts and ethanol

To perform its probiotic effect, it is necessary that the bacteria or yeast reaches the host's gastrointestinal tract at high enough levels to play this role. Therefore, it is necessary that a probiotic is tolerant to oxygen, acid media, bile and heat. The standards show that probiotic products must have at least 10^6 viable microorganisms per millimeter (Stack *et al.*, 2010; Franz & Holzapfel, 2011; Zoumpoulou *et al.*, 2017).

S. boulardii reached values higher than 10^6 UFC mL⁻¹ in all the tested pH values, as shown in Table 1. The results demonstrate that during the first 30 min of exposition, the counting grew in all tested pH strips. A study with *Lactobacillus acidophilus* NIT reported that, in 60 min of exposition to pH 2, there was a 20% survival rate of the bacteria. However, this number dropped to an undetectable level in 120 min. On the other hand, on pH 3, the percentage of survival was larger in the entire process (Pan *et al.*, 2009).

Table 1. *S. boulardii* count after exposition to environment acidified with HCl and bile salts for 30, 60 and 120 minutes.

Time (min)	<i>S. boulardii</i> count (Log UFC mL ⁻¹)					
	pH			Bile (%)		
	1.0	2.0	3.0	0	0.2	0.3
30	6.63	7.59	7.77	7.96	7.43	6.91
60	6.79	8.05	8.03	8.72	8.05	7.30
90	7.04	9.05	9.05	9.77	9.46	8.46

Source: Authors

The capacity to resist bile is one of the most important properties that determine if the bacteria can survive in the small intestine and, consequently, its probiotic potential (Bustos *et al.*, 2018). The data presented on Table 1 show that the analyzed strain presented resistance to bile salts, with a decrease of only one logarithm cycle after the 90 min exposition to bile salts at 0.2 and 0.3%. Andriantsoanirina *et al.* (2013) studied the resistance of different species of *Bifidobacterium spp* and observed that *B. breve* and *B. adolescentis* presented the highest number of moderately bile-resistant strains. Besides that, another study reported that *Lactobacillus* isolates have resistance higher than 50% in concentrations of 0.15, 0.3 and 0.45% of bile salt (Kumar & Kumar, 2015).

The results demonstrate that the *S. boulardii* strain's growth was proportional to the increase of the ethanol concentration, being the values 14.27, 14.69, 15.48, 17.27, 17.69 Log UFC mL⁻¹ at 1, 2, 3, 4 and 5% of ethanol, respectively. These results confirm the *S. boulardii* resistance in the conditions studied. Stewart (2014) affirms that one of the main characteristics of the *Saccharomyces* is its high tolerance to high concentrations of ethanol. In addition to that, the author highlights that the resistance of different strains of *S. cerevisiae* may vary, but the largest majority is able to grow in beverages with 8 to 12% alcohol (v/v).

Finally, the *S. boulardii* strain presented probiotic viability after confirmation of the resistance to acid, bile salts and ethyl alcohol. Therefore, this strain is a candidate to probiotic cultures in alcoholic fermentation products.

3.3 Analysis of the beer's quality standards

The beer produced with probiotics had similar visual qualities to Pilsen craft beers, which is due to the fact that neither has been through the filtering process after the fermentation and both contained cells in suspension. Results of the counting of *S. boulardii* during a 28-day storage, as demonstrated in Table 2, have shown decreasing values until the 21st day of storage, with the reduction of about one logarithmic cycle on the final counting of time. All the obtained values are considered satisfactory for the product to be considered probiotic because, according to Nag & Das (2013), the minimum number of probiotic bacteria at the moment of ingestion must be $\geq 10^6$ UFC g^{-1} in order to achieve therapeutic effects. A study of supplementation of soy yoghurt with extracts and mulberry leaves shows that, after 30 days, the quantities of *L. casei* 01 and *L. acidophilus* LA5 stayed above 6 \log_{10} UFC g^{-1} (Kemsawasd & Chaikham, 2018).

Table 2. Counting of probiotic beer during a 28-day storage time.

Days	<i>S. boulardii</i> count (Log UFC mL^{-1})
1	14.22
7	17.27
14	19.40
21	20.89
28	19.69

Source: Authors

For comparison purposes, physicochemical analysis was carried on the beers with and without the addition of probiotic culture and the results were expressed in Table 3. The alcohol content of the samples varied between 4.70 and 6.06%, being superior to the ones reported by Ceccaroni *et al.* (2018) for gluten-free rice-malted beer (4.80%). Among the parameters demanded by legislation, the alcohol content is the only one that must be declared on the product's label (Brasil, 1997).

The samples of beer with and without the addition of probiotics had pH values of 4.57 and 4.60, respectively. These values are similar to the ones reported by Ramsey *et al.* (2018) for beers with different percentages of alcohol (4.17 to 4.20). Most beers present pH between 3.9 and 4.6 and their safety is connected to that as well as to the presence of ethanol, anaerobic conditions and the presence of antimicrobial agents, such as iso- α -acids (Bamforth, 2008). The average acidity of the samples varied from 1.6 to 2.0 g of lactic acid by 100 mL of sample. In the Brazilian legislation, there is no mention of maximum permitted acidity or pH for beer.

Table 3. Physicochemical analysis of beers with and without addition of probiotic culture after a 28-day period of storage

Analysis	Specification	Beer without probiotics	Beer with probiotics
Alcohol	% v v ⁻¹	5.79	6.06
Volatile acidity	m Eg L ⁻¹	1.60	2.00
Dry defatted extract	g 100 mL ⁻¹	5.25	5.03
Total extract	g 100 mL ⁻¹	5.25	5.03
Protein	g 100 mL ⁻¹	0.48	0.40
Fixed mineral residue	g 100 mL ⁻¹	0.16	0.14
pH	g 100 mL ⁻¹	4.57	4.60
Primitive extract	°P	14.67	14.49
Real extract	°P	6.06	5.45
Apparent extract	°P	4.07	3.36
Limit extract	°P	2.93	2.83
Fermented extract	°P	1.14	0.53
Color	EBC	8.60	9.00
Bitterness	BU	7.00	7.50
Acetaldehyde	mg L ⁻¹	8.10	9.80
Ethyl acetate	mg L ⁻¹	16.40	9.60
Isoamyl acetate	mg L ⁻¹	1.28	0.39
Hexanoate of ethyl	mg L ⁻¹	0.23	0.25
Total superior alcohols	mg L ⁻¹	89.50	96.80
DMS	µg L ⁻¹	16.00	15.00
Free diacetyl	µg L ⁻¹	39.00	45.00
Total free VDK	µg L ⁻¹	53.00	52.00
µg L ⁻¹	4.00	4.00	

Source: Authors

Values for the color parameter of the beers with and without the addition of probiotics were obtained. They were respectively 8.6 and 9.0 EBC, being a little darker when compared to Lager beers submitted to the ultrasound treatment (3.6 a 4.5 EBC) (Deng *et al.*, 2018). The beers are classified by the Brazilian legislation as light-colored (EBC<20) or dark (EBC>20) (Brazil, 1997). Deng *et al.* (2018) even obtained a bitterness interval of 10.28 to 11.42 BU, the beers from this study being the most bitter (7.0 to 7.5 BU). In order to provide aroma and bitterness to the beer, hops are added to the wort and this

process leads to the formation of compounds considered to be the main source of bitterness of the beer as, for example, the iso- α -acids. Therefore, the intensity of bitterness is due to the level of iso- α -acids present in the beers (Schönberger & Kostecky, 2011).

Amongst all the aromatic compounds that can dramatically contribute to the beer flavor, the esters and superior alcohols are the ones found in the largest quantities. Both beers with and without the addition of probiotic culture presented total superior alcohol values of 89.5 and 96.8 mg L⁻¹, respectively. Moreover, esters like ethyl acetate, isoamyl acetate and ethyl hexanoate provide fruity flavor to the beer (He *et al.*, 2014). The rate of acetaldehyde of both samples was high, which might be related to the fact that the beer having incorporated little oxygen after the inoculation stage.

Concerning the real extract, beers with or without the addition of probiotic culture had percentages of 6.06 and 5.45% respectively. These percentages were lower to the ones reported by Cimini & Moresi (2018) for two beers of different types (3.90 and 5.80%). These differences might possibly be explained by technological variations, such as a greater use of malt.

3.4 Sensorial Analysis

The results of the sensorial test, expressed in the Table 4, showed that the beer with the addition of probiotics had superior averages than the beer without probiotics in the attributes flavor, aroma and global appearance, not differing from the commercial beer ($p < 0.05$). These results show that the craft beer produced without the addition of *S. boulardii* had the lowest acceptance by the consumers. This is probably due to the production of undesirable aromas by the low-fermentation yeast. As for the body and acidity attributes, the craft formulations (with or without probiotics) registered inferior averages than the ones obtained for the commercial beers, which might be related to the fact that they are a more familiar product to the tasters, presenting more pleasant characteristics in these parameters.

Table 4. Sensorial analysis for craft beers with and without probiotics and for commercial beer.

Attribute	Beer without probiotics	Beer with probiotics	Commercial beer	Tukey's MDS
Flavor	5.08 ^a	6.12 ^b	7.00 ^b	0.98
Aroma	5.18 ^a	6.88 ^b	6.82 ^b	1.02
Color	5.92 ^a	6.47 ^a	6.53 ^a	0.97
Body	5.35 ^a	6.29 ^a	6.41 ^b	0.97
Global appearance	5.65 ^a	6.67 ^b	6.94 ^b	0.88
Acidity	5.25 ^a	5.71 ^a	6.73 ^b	0.96

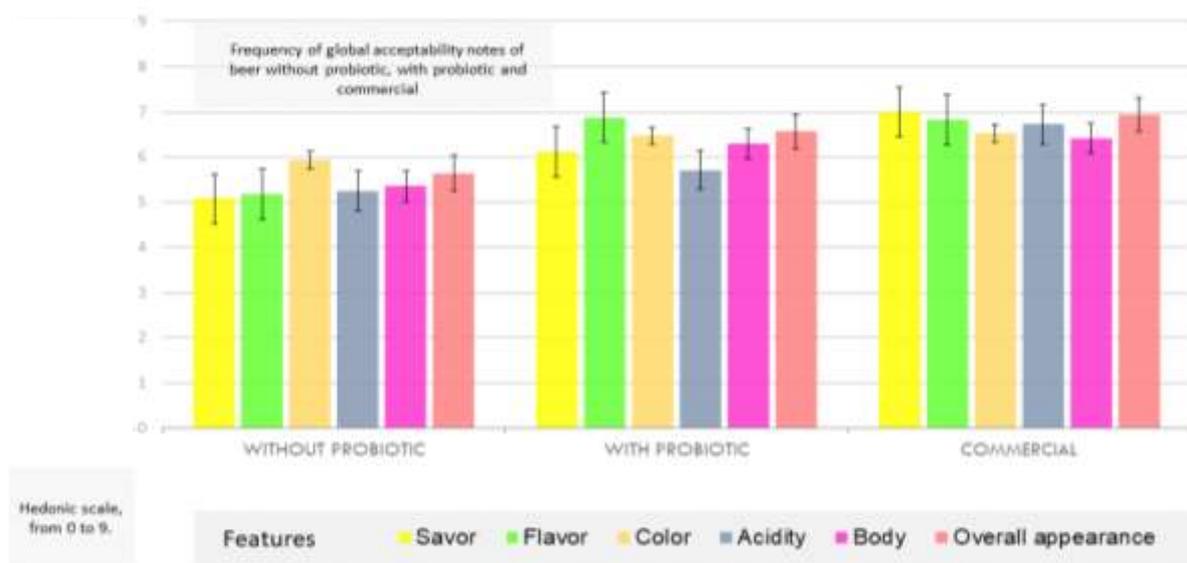
*Averages followed by the same letter (on each line) have not differed statistically ($p > 0.05$). Source: Authors.

Among the sensorial parameters evaluated (Table 4), only the attribute of color had no difference among the samples ($p > 0.05$), which shows that the beers have not presented enough color difference to be noticed by the tasters, probably because they were all produced from the same type of wort.

The acceptance averages varied from 5.08 (without probiotics) and 7.00 (commercial), with “indifferent” and “liked moderately” being in the hedonic points. Di Monaco *et al.* (2004) suggests that this behavior happens when the tasters are not familiar with the samples' identity, not being able to tell the difference between the verified samples thus establishing their preference in the intermediary part of the scale.

Figure 3 shows the frequency of grades obtained in the acceptance test for the beer samples. It can be observed on the histogram that the commercial beer was the one which presented the highest frequency of grades on the positive side. However, the beer with the addition of probiotic culture obtained near averages for the same attributes.

Figure 3. Frequency of global acceptance grades of the beer without probiotics, with probiotics and commercial with a hedonic scale of 9 points. Based on the results, it can be said that the probiotic culture has particular properties which allow it to be used for the production of a quality Pilsen-style beer.



Source: Authors

4. Conclusion

The probiotic culture of *Saccharomyces boulardii* have was able to survive in the studied conditions of resistance (pH, bile acids) and its use to beer production is in accordance to the expected physical-chemical and microbiological standards. Besides that, the viability of the probiotic culture was verified for 28 days, with a cell count superior to 10^6 UFC g^{-1} . The sensorial analysis showed a good acceptance level for the produced beer, especially for the attribute's aroma, flavor, global appearance and color. The obtained results have pointed out that the beer with the addition of probiotic culture presents viability for industrial scale production. Further studies could be conducted to know how the regular consumption of probiotic beer affects the consumer's health.

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