

## Evaluation of physical-chemical and microbiological characteristics and sensory profile of pilsner beers sold in Serra Gaúcha

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### Abstract

**Objective:** To evaluate and compare physicochemical, microbiological, and sensory characteristics of *Pilsen* beers marketed in Serra Gaúcha. **Methods:** In the first study, 11 samples, with the *Pilsen* denomination on the label, were submitted to physicochemical, microbiological, and organoleptic tests. In the second comparative, five people were identified and selected as appreciators (Group A) and five people were identified and selected as non-appreciators (Group B). **Results:** The item flavor showed statistical significance between Group A and the reference beer. In the physicochemical analyses, there was a statistical difference ( $p \leq 0.01$ ) in color between the reference and Group B, which also presented a higher than average alcohol content as compared to the others. As for the microbiological analyses, one sample presented growth of *Brettanomyces sp.* It is therefore possible to verify that deficient standards exist in the manufacturing process of the products where the inclusion of additives is allowed, regardless of origin, which ultimately confuses and hinders the consumers' perceptions of quality in the product. **Conclusion:** More rigid legal parameters are suggested and new production techniques are needed to generate more differentiated and higher-quality products. Finally, a clear legal deficiency was observed in the brewing sector.

**Keywords:** Beer, *Pilsen*, brewing industry.

## Avaliação das características físico-químicas e microbiológicas e perfil sensorial de cervejas pilsen vendidas na Serra Gaúchs

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## Resumo

**Objetivo:** Avaliar e comparar as características físico-químicas, microbiológicas e sensoriais das cervejas Pilsen comercializadas na Serra Gaúcha. **Métodos:** No primeiro estudo, 11 amostras, com a denominação Pilsen no rótulo, foram submetidas a testes físico-químicos, microbiológicos e organolépticos. Na segunda comparação, cinco pessoas foram identificadas e selecionadas como apreciadoras (Grupo A) e cinco pessoas foram identificadas e selecionadas como não apreciadoras (Grupo B). **Resultados:** O item sabor mostrou significância estatística entre o Grupo A e a cerveja de referência. Nas análises físico-químicas, houve diferença estatística ( $p \leq 0,01$ ) na cor entre a referência e o Grupo B, que também apresentou um teor alcoólico acima da média em comparação com os outros. Quanto às análises microbiológicas, uma amostra apresentou crescimento de *Brettanomyces* sp. É possível verificar que existem padrões deficientes no processo de fabricação dos produtos onde a inclusão de aditivos é permitida, independentemente da origem, o que acaba confundindo e dificultando as percepções dos consumidores sobre a qualidade do produto. **Conclusão:** Parâmetros legais mais rígidos são sugeridos e novas técnicas de produção são necessárias para gerar produtos mais diferenciados e de maior qualidade. Por fim, observou-se uma clara deficiência legal no setor cervejeiro.

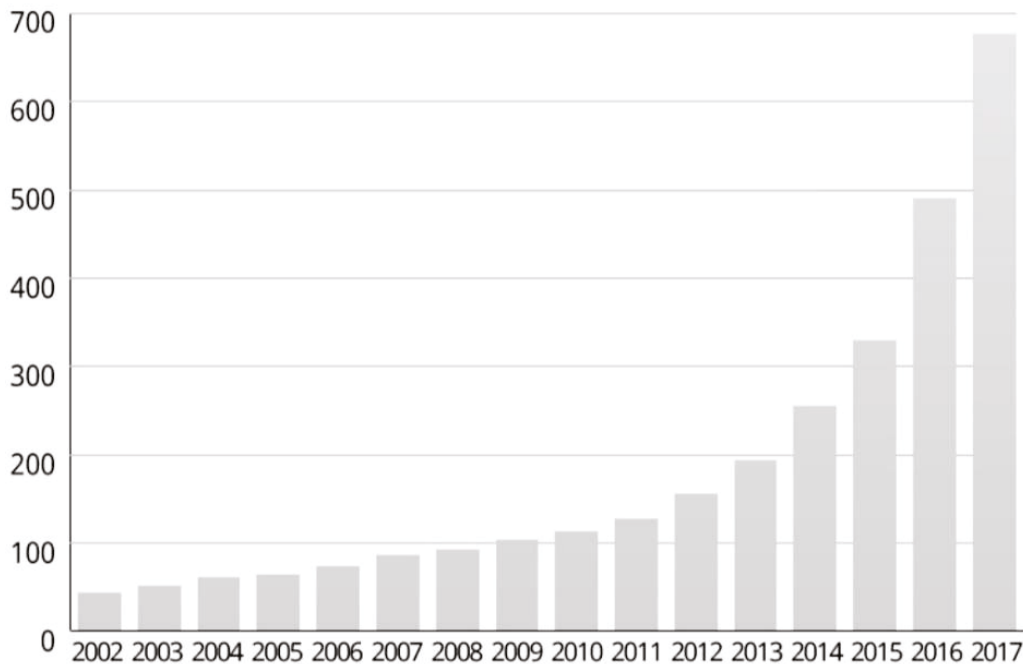
**Palavras-chave:** Cerveja, Pilsen, indústria cervejeira.

## Introduction

The beer market in Brazil has shown significant growth, and the structures of the beer industry are highly concentrated and characterized by the presence of leading companies and brands. According to data obtained by

the Brazilian Craft Beer Association, Abracerva, in 10 years, Brazil has gone from 70 to 700 craft breweries with regularized registration, as shown in the graph in Figure 1 (Ramos, 2019).

Figure 1 – Number of Craft Breweries in Brazil



Source: MAPA (2018)

The macro-breweries, which serve the entire national territory, are formed by the Medium and Large Brewing Companies (MGE) and are responsible for the largest share of beer consumption among the population and the largest production in terms of volume. The microbreweries, which are incorporated into the Micro and Small Brewing Companies (MPE), respond to preliminary local demand, and are characterized by low production volumes and lower installed production capacity. However, as published by MAPA in 2020, 204

new breweries were registered in Brazil and another 30 canceled their registrations, which represents an increase of 174 breweries, 14.4% compared to the previous year (Sgorla, A. F., 2023).

The current definition of beer production in Brazil follows rules defined by legislation, which specifies that beer originates from the alcoholic fermentation of wort, derived from barley malt and drinking water and the action of yeast intermingled with hops (SEBRAE, 2017).

Any modification to this process and in the raw material can result in beers with different physicochemical and sensory characteristics. In mid-2014, a survey conducted by the Barth Haas Group showed that Brazil is the third largest beer producer worldwide behind only China and the United States, having produced approximately 14.04 billion liters in 2014. Years later, Brazil is still the third largest beer producer in the world (Ramos, 2019; Lima J. D. M., 2023).

The amount of alcohol depends very much on the fermentation process. The production and consumption of the majority beer in Brazil comes from *lager pilsener*, which is a low fermentation beer fermented by *S. pastorianus*. This yeast is a hybrid of *S. cerevisiae* and *S. eubayanus*. It is characterized by mild flavor, pH around 4.3, clear and limpid (Pereira, 2020). During fermentation, the sugars transform into alcohol, heat and CO<sub>2</sub>, a combination that results in a carbonated alcoholic beverage (De Arruda, Junior and Goulart, 2013). In Brazil, *Pilsen* beer is less full-bodied, with reduced bitterness and low alcohol content, thus being lighter and more refreshing. Such measures are adopted as standard by major national brands, thus performing a mixture of styles between *Pilsen*, *Lager* and *Lager Beer* (Beltramelli, 2012; Scheffer et al., 2013).

Historians believe that craft beer has existed for centuries when bread was fermented in water (Beltramelli, 2012). Presently craft beers are judged to be part of a class of products derived from superior quality and higher added value. There are elaborate means of formulation and processes distinct from those products created on an industrial scale. Small-scale production enables the development of differentiated products for the most exquisite consumers, with emphasis on valuing the selection of regional raw materials for product creation. (CERVBrasil, 2019).

For the characterization and identification of the considerable compounds for beer quality, physicochemical and sensory methods are widely applied. Sensory methods are grouped into three streams: affective, discriminative, and descriptive tests. Affective tests aim to objectify whether the product is accepted or preferred by the consumer. In discriminative tests, researchers analyze if the subjects can discern a perceptible difference between the products. In descriptive tests, researchers seek to determine the main relevant points between the products studied and their intensities (Dutra, 2019).

Given the growth in consumption in the sector and the large number of brewing companies, the study is necessary to evaluate the physicochemical, microbiological, and sensory characteristics, aiming to assess the products available to consumers in Serra Gaúcha.

## Methods

This is a comparative analytical study, performed in two stages. The first study was elaborated with 11 *Pilsen* beers: five of the craft type, five of the industrial type, and one from the Czech Republic. The products were purchased between November 1, 2016 and November 5, 2016 in local commerce. The main inclusion criterion the characterization of *Pilsen* type beer on the respective labels.

The sensory evaluation was performed through a descriptive method, which establishes qualitative and quantitative differences in each type of beer. Evaluations are performed using attribute and proportion scales. Odor and taste, texture, appearance, flavor and texture properties are described. The relationship between the duration and intensity of a stimulus is measured.

It was performed by ten individuals of both sexes, aged between 22 and 38. Of the group members, five had a university degree in oenology, with technical knowledge of brewing. The remaining five, considered self-proclaimed connoisseurs, had no formal training in the area.

The sensory analysis was performed through tasting in an appropriate room with all the necessary items for the tests. The samples were uncharacterized, wrapped in a black bag in order to make it impossible to identify their commercial brand, and served one by one to each participant, with approximately 100mL at a temperature of 2°C in a glass suitable for tasting. After tasting each beer, the participants filled out a beer evaluation form, adapted from BJCP *Beer Scoresheet*, 2009, which was collected at the end of the tastings.

In the physical-chemical analysis, the procedures for pH, volatile acidity, turbidity, color, density, alcohol, total dry extract and total acidity were performed. We used methodologies from the Beverage and Vinegar Operational Manual (IN 24/2005), from the alcoholic fermented products section, being the methods two, three, four, six and eight for density, alcohol, pH, volatile acidity and total dry extract, respectively. For total acidity, turbidity and color analysis, methods MA-AS3113-01 from the World Organization of Vine and Wine, OUGH C.S, *Methods Analysis of Musts and Wines* and *Analytica EBC Method* 9.6 were consulted, respectively.

The samples were also submitted to three microbiological tests: identification of aerobic mesophilic microorganisms, by the *Plate Count Agar* technique, *Compendium of Methods for the Microbiological Examination of Foods*; mold and yeast, by the DRBC Agar technique - APHA 4th Edition, *Compendium of Methods for the Microbiological Examination of Foods*; and *Brettanomyces sp.* by the plating/selective medium technique - Millipore.®

In the second study, 10 beers were purchased, 5 craft *Pilsen* and 5 industrial *Pilsen*. Ten individuals were invited to participate in the tasting, five of whom were beer lovers, i.e., individuals who maintain the habit of consuming beer with a certain frequency, and five others who were not beer lovers. In an appropriate room, 10 samples of totally uncharacterized beers were placed so that the individuals could identify, through tasting, whether the beer was craft or industrial, through sensory analysis of the product. The same descriptive method was applied.

In order to ensure a reliable result, the analyses were performed with the help of a laboratory accredited by the CGCRE, with vast experience in beverage analysis. To conclude the results, it was necessary to apply the

T-test for mean and standard deviation. The software used for statistical analysis was SPSS 20.0 for Windows.

## Results and Discussion

The results of the physicochemical analyses of the beers are presented in tables 1 and

2. The evaluation of the sensory profile is presented in the form of a table, numbers 3 and 4. We can observe that there were no significant results among the factors analyzed ( $p < 0.05$ ). The analyses, provided by a laboratory with INMETRO/Cgecre accreditation, were made after calibrating the equipment, as well as reading the control sample, with previously known values, and the “blank” sample, in order to verify the accuracy and precision of the results.

Table 1 – Physical-chemical analyses of beers from macro breweries (Group A), beers from micro breweries (Group B) and beer from the Czech Republic (B6)

Samples	pH	Volatile Acidity (meq/L)	Turbidity
A1	4,62	0,276	00,60
A2	4,35	0,246	00,41
A3	4,32	0,246	00,86
A4	4,19	0,198	01,06
A5	4,37	0,294	00,69
B1	4,65	0,240	59,70
B2	4,47	0,366	03,60
B3	4,86	0,192	19,90
B4	4,79	0,186	13,20
B5	4,63	0,114	29,20
B6	4,75	0,190	01,80
<b>Mean ± SD</b>	4,55 ± 0,218	0,37 ± 0,125	11,91 ± 18,562

Values expressed as Mean ± Standard Deviation (SPSS 20.0 for Windows); \*  $p < 0.05$ .

In the samples analyzed, five beers, four industrial and one handmade, showed pH values below 4.5, which makes them unsuitable for consumption as pH values up to this range are of fundamental importance, because they inhibit the development of pathogenic microorganisms, especially *Clostridium botulinum*, the bacterium that causes botulism, and thus prevent further contamination. Beer contamination can occur due to several factors, and in addition to the performance of laboratory tests, in many breweries sensory tests are also performed (Nunes, 2021).

The acidity in the product is due to the formation of acids during fermentation; the main organic acids formed are: acetic acid, formic acid, pyruvate, D-lactate, L-lactate, among others. Thus, it is of utmost importan-

ce to perform a strict quality control to guarantee the standardization of the beer and avoid losses and waste during the production process (Nunes, 2021). Regarding microbiological analysis, only one microbrewery sample showed growth of *Brettanomyces sp.* with 1 CFU/mL.

In the second test, composed of a group of lovers and a group of non lovers, we can see that there was also no significant difference between them, as shown in table 5.

Table 2 – Physical-chemical analyses of beers from macro breweries (Group A), beers from micro breweries (Group B) and beer from the Czech Republic (B6)

Samples	Color (EBC Units)	Density at 20°C	Alcohol (% vol.)	Dry Extract (g/L)	Total Acidity (meq/L)
A1	7	1,00830	4,56	38,3	22,10
A2	5	1,01000	4,71	43,4	19,50
A3	6	1,00950	4,70	42,1	21,20
A4	5	1,00950	4,40	41,1	17,00
A5	7	1,00768	3,85	34,5	17,40
B1	7	1,01243	4,80	49,6	18,90
B2	10	1,01287	5,13	51,9	24,40
B3	12	1,00840	4,74	39,4	16,20
B4	7	1,01040	4,83	44,7	16,40
B5	12	1,01076	4,62	44,7	21,10
B6	10	1,00890	4,82	40,1	16,40
<b>Mean ± SD</b>	7,75 ± 2,541	1,01 ± 0,002	4,65 ± 0,322	42,71 ± 4,980	19,14 ± 0,276

Values expressed as Mean ± Standard Deviation (SPSS 20.0 for Windows); \* p<0.05.

Table 3 – Comparison of sensory analyses of winemakers between beers produced in macro breweries (Group A), beers produced in micro breweries (Group B) and beer from the Czech Republic (B6)

Criterion	Group A		Group B		Group B6		P
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation	
Appearance	2,92*	0,76	3,84*	0,75	3,20	1,79	<0,01*
Aroma	4,40*	2,25	6,68*	1,55	5,60	3,21	<0,01*
Flavor	4,92*	2,22	6,24	2,33	7,20*	1,48	0,04*
<b>Palate</b>	3,08	1,50	3,80	1,41	3,40	2,07	0,25

Values expressed as Mean ± Standard Deviation (SPSS 20.0 for Windows); \* p<0.05.

Table 4 – Comparison of sensory analyses of non-enologists between beers produced in macro breweries (Group A), beers produced from micro breweries (Group B) and beer from the Czech Republic (B6)

Criterion	Group A		Group B		Group B6		P
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation	
Appearance	3,56	0,87	3,80	0,96	4,00	0,71	0,49
Aroma	7,26	1,79	7,04	2,92	6,20	3,56	0,69
Flavor	7,10	1,19	7,16	2,54	8,20	1,30	0,50
<b>Palate</b>	3,48	1,19	3,20	1,55	3,20	1,92	0,77

Values expressed as Mean ± Standard Deviation (SPSS 20.0 for Windows); \* p<0.05.

Table 5 – Differentiation of industrial craft beers into connoisseurs (Group A) and non-connoisseurs (Group B)

	Group A (Appreciators)		Group B (Non-Appreciators)	
	Hits	Errors	Hits	Errors
<b>Total</b>	<b>35</b>	<b>15</b>	<b>31</b>	<b>19</b>

Values expressed as Mean  $\pm$  Standard Deviation (SPSS 20.0 for Windows); \*  $p < 0.05$ .

Thus, the groups had difficulties in differentiating craft beer from industrial beer. In view of this, it is possible to see a lack of standard in the manufacturing of products, where the use of various additives is allowed or not, stages such as mashing, fermentation, maturation and the use of different ingredients such as wheat, corn, rye, rice, honey, fruit and others that can change the taste of the product. Regardless of the origin, which ends up masking the organoleptic characteristics, confusing and damaging the consumers' taste.

The old German Beer Purity Law (*Reinheitsgebot*), dating back to 1516, states that beer should be produced without any adjuncts, exclusively with malt, hops and water. Brewer's yeast should not be considered as a raw material, being necessary only as a biochemical agent in the transformation of sugars into alcohol by the fermentation process. This law established the quality of the ingredients, not the manner in which they should be processed. (Dias, 2018).

The physicochemical criteria are important instruments used by the legislation as a way to control and evaluate the production processes as seen in Tables 1 and 2. Since it influences many factors, such as microbial growth, color intensity, enzyme activity, oxy-reduction potential, and also flavor, it should be kept below 4.5 (Dutra, 2019).

The alcohol content was higher in the microbrewery groups, with an average of 4.82%, and in the beer of Czech origin, with 4.65%. Regarding color, according to Article 38 of Decree No. 6871 of June 4, 2009 (Dutra, 2019), beer can be classified as light beer, which has a color corresponding to less than twenty EBC units (*European Brewery Convention*). All analyzed samples were classified as light, characteristic of *Pilsen* beers. A study that analyzed the information on the labels of *Pilsen* beers found that all the brands studied were in accordance with current labeling standards (Almeida, 2015).

Since beer is defined as the favorite among Brazilians (IBOPE, 2013), it would be expected that beer lovers would identify different characteristics when compared to products from micro or macro breweries, as well as when compared to a beer from the Czech Republic, the birthplace of *Pilsen beer*. The lack of identification of differentiated sensory characteristics among beer lovers demonstrates the need for improvements in the perceptive capacity of this group, which can often end up consuming

a product through advertising trends (Della Lucia et al., 2013), price, or even the maturity of sensory evaluation.

Through the evaluation of the sensory profile by the groups, one can see that higher education in the area is important in the differentiation of parameters such as appearance and aroma, mainly. A similar study observed that, when comparing two *Pilsen* type beers produced in micro breweries with two market-leading *Pilsen* type beers, the micro breweries' beers have greater intensity in the sensory attributes, differentiating themselves in color, yeast aroma, sweet taste and bitter taste. However, there was no statistical difference between these evaluators, contrary to what was expected, because micro breweries develop a beer with more artisanal characteristics and that should provide greater attraction to the consumer (Minim e Da Silva, 2016).

For further testing, regarding technical matching for differentiating samples appropriately about consumer perception, the Check-All-That-Apply (CATA) method can be used. It involves the use of agreeability scales as well as the characteristics attributes to differentiate a product. The supplied scale to consumers can be used to design an internal preference map, in which it is possible to visualize consumer response towards products. Using narrative attributes provided by a trained panel, along with the consumer's preference map, can be used to create an external preference map, which can help understand which attributes are important in relation to consumer preferences (Lee, 2023).

## Conclusion

Regarding the microbiological evaluation, of the eleven samples used, ten were not contaminated, while the missing sample showed the presence of *Brettanomyces sp.* with 1 CFU/mL; probably the presence of this yeast in the sample was not able to influence the sensory characteristics of the samples, however, its presence should be monitored because this microorganism is the main responsible for causing turbidity, odors, and undesirable flavors in the final product.

Even though the microbrewery market is on the rise and their products are reaching a larger portion of the beer consumption in Brazil, they must explore characteristics of the production process that aim to positively surprise the consumers' sensory evaluation. As proof of this, during the two tests performed with the

group without technical knowledge (which represents the largest part of the beer consuming population) they were unable to identify the differences between a craft product, characterized by the use of higher quality raw materials, and an industrial product, commonly known for using other raw materials in order to lower production costs. Thus, these data provide us with the understanding that the choice of product occurs by factors that go beyond the

organoleptic experience and are closer to an economic, cultural, visual issue, by indication of friends or family, and mainly by the influence of the media. During the development of this research, the legal deficiency in the brewing industry was also identified, since there is little legislation regarding manufacturing and quality control, causing difficulty in production standardization and generating gaps in the effectiveness of product surveillance.

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