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PHYSICAL-CHEMICAL AND MICROBIAL QUALITY OF THE "CERVEJA CASEIRA" (HOMEBREWED BEER) OF GUARAPUAVA/STATE OF PARANÁ – BRAZIL

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Abstract

"Cerveja caseira" is a homebrewed beer sold in the Guarapuava municipality (State of Paraná, Southern Brazil). It was introduced by Slavic immigrants and adapted to Brazilian ingredients, being much appreciated in the region and disseminated by popular knowledge. The objective of this research is a physical-chemical and microbial analysis of cerveja caseira produced and sold in Guarapuava. Fifty-six samples divided in 14 lots were acquired in October and November 2020 in streets, houses, commerce, and markets of Guarapuava. The physical-chemical analyses included the determination of the following parameters: color, pH, original extract, apparent extract, alcohol, density, bitterness, SO_2, and calories. The microbial quality was assessed via counts of total aerobic mesophiles, total and thermotolerant coliforms, positive-coagulase Staphylococcus, Pseudomonas spp, Bacillus spp, molds, and yeasts. The physical-chemical results indicate the lack of standards for the cerveja caseira production, with ample variations regarding color, original and apparent extract, alcohol percentage, and bitterness. Regarding microbial quality, all cerveja caseira lots contained pathogens, mainly mesophiles, yeasts, and Staphylococcus aureus, but no total and thermotolerant coliforms, important contamination indicators. According to Brazilian beer-related regulations, only lot 4 could be commercialized, despite its high percentage of alcohol (2.5% v/v), which characterizes it as an alcoholic beverage, not suitable for consumption by children and adolescents. Cerveja caseira recipes have been passed on from generation to generation, adapted to the climate and to the products available in Brazil, being part of the cultural heritage of the western region of Paraná that should be preserved.

Keywords: artisanal drink, non-alcoholic beer, Slavic culture, microbial contamination

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1. Introduction

The region of the Guarapuava municipality (State of Paraná, Southern Brazil) has experienced social and economic changes, thanks to the arrival of Slavic immigrants from 1890 and 1914. The State of Paraná welcomed a large number of Polish and Ukrainian immigrants, who searched for better life conditions in Brazil (TELEGINSKI, 2014).

In Paraná, immigrants tried to preserve their identities, including food habits, even though these are some of the last elements to cause denationalization or losing the reference of the culture of origin, constituting one of the foundations for the feeling of belonging in the contact with the "other". (DUTRA, 1991).

Until today, many Slavic descendants have cultivated and prepared food in their traditional manner, the so-called "typical dishes", accompanied by artisanal drinks, such as *cerveja caseira* (*pivo* in Polish or *pevo* in Ukrainian), and also *gengibirra* (ginger soft drink or beer) (GRECHINSK; CARDOSO, 2008).

Man's passion for beer – also called "liquid bread" – permeates humans' history, being part of the daily life of many civilizations. The practice of beer making appears to have originated in Mesopotamia, where barley grows wild (AQUARONE; BORZANI; SCHMIDELL, 1986). Evidence of its manufacture in Babylonia dates back to 6000 BC. In Brazil, the European habit of drinking beer was introduced by D. João VI in the beginning of the 19th century (VENTURINI FILHO, 2016).

Beer contains hops, water, barley, and yeast, in addition to brewing adjuncts, and it is the quantities of these ingredients, the way they are processed, and the duration of the manufacturing stages that characterize the various types of beer produced in breweries (AQUARONE; BORZANI; SCHMIDELL, 1986).

According to Campigoto, Slominski, Schörner (2014), the production and consumption of beer is part of the Slavic culture. *Cerveja caseira* is a non-alcoholic drink served to people of all ages, family, friends, and work meetings, and is produced by the consumers themselves or bought from neighbors, relatives, or even in informal markets. *Cerveja caseira* can be considered a cultural movement that transcends our modern "fast food" or "fast beer" environment.

In Guarapuava, *cerveja caseira* is usually produced by women (mothers, daughters, sisters, and wives). The family men are in charge of commercializing it in streets, houses, grocery stores, and free markets (producer's market).

In 1997, Cervejaria Krulowa® of Irati (State of Paraná) requested the licensing of the *cerveja caseira* commercial-scale production from the *Ministério da Agricultura, Pecuária e Abastecimento* (Ministry of Agriculture, Livestock and Supply – MAPA). Because barley is not used in the *cerveja caseira* production, the term "fermented hop beverage", rather than "beer", should be applied to it. Its production was discontinued in the early 2000's, due to competition with *tubaínas*, among other reasons (GARDIN, 2020).

The *cerveja caseira* recipe, according to Campigoto, Slominski, Schörner (2014), includes hops, sugar, water, yeast, and egg white, and sometimes cloves and ginger. At present, however, some producers decided to avoid egg whites, as some customers may be allergic to them.

Children and the elderly are the people who appreciate the Guarapuava *cerveja caseira* the most, as its said to be a non-alcoholic beverage. This public is also attracted to it for the great variety of recipes. Besides the light *cerveja caseira*, there are the "darker" types, which result from the use of dyes or different points of sugar caramelization; some are produced with the addition of fruits, *e.g.* pineapple. The color and the flavor of *cerveja caseira* made with fruits are changed, and recipes and ways of producing it vary, indicating that the popular culture is a mutant phenomenon.

The container used for bottling has also changed along the years. *Cerveja caseira* was once kept in large barrels, but today plastic bottles are used, as they better withstand the effects of brewing by dilation.

The majority of the *cerveja caseira* producers live in the rural area and subsist on the sales of agricultural products. The water used to produce *cerveja caseira* is obtained from artesian wells. When not grown in their properties, hop is bought in Guarapuava markets.

The historical-cultural importance of this activity is depicted from conversations with producers, who associate the freshness of *cerveja caseira* with family meetings and important moments in their lives. The recipe used can be considered a knowledge that has very proudly and satisfactorily passed on from generation to generation. Besides, according to Asquieri et al. (1997), the production of *cerveja caseira* can be helpful to small rural producers by increasing the family income and adding value to it.

Beer contamination can result from many factors and, besides laboratory tests, sensorial tests – that is, the tasting of the produced beer – are also performed in breweries. Smell is an extremely important sense to identify possible changes and contamination in beer. Aroma and intensity can vary in beer according to the type of bacteria or wild yeast, causing flavors and off-flavors. Acidity and bitterness also indicate that the beer is contaminated. Besides, contaminated beer can also present unwanted visual characteristics (ALEGRE, 2014).

The objective of this research is to present a physical-chemical and microbial analysis of the *cerveja caseira* produced and sold in Guarapuava (State of Paraná, Southern Brazil).

2. Material and methods

2.1 Material and methods

The city of Guarapuava is located in the central-southern portion of the State of Paraná (Figure 1). It is 247 km far from the capital Curitiba, 361 km from the Paranaguá port, and 389 km from the triple frontier of Foz do Iguaçu (FERREIRA, 2010), at an altitude of 1,120 m (PFANN et al., 2009).

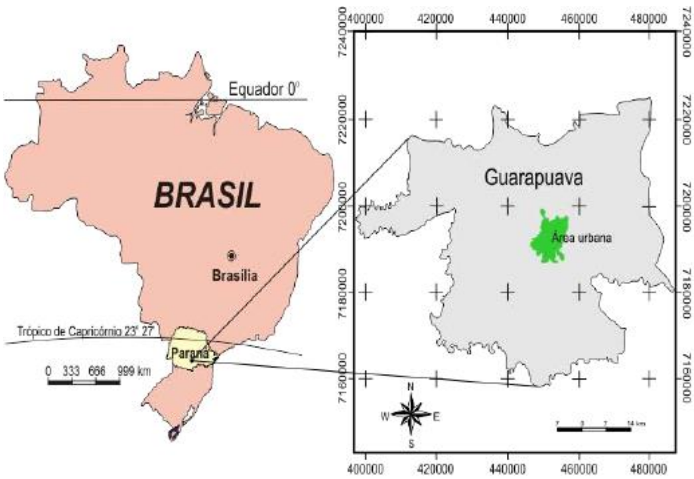

Figure 1. Location of the Guarapuava urban area in the State of Paraná (Southern Brazil).

Source: Gomes and Vestena (2018)

Guarapuava is one of the coldest municipalities of the State of Paraná, with occasional snow falls. The predominant biome is the Atlantic Forest, which is mainly composed of the phytophysiognomic formation named Mixed Ombrophilous Forest or Araucaria Forest (MIRANDA; ZAGO, 2015). It is also the largest Brazilian barley producer and has the largest malt factory in Latin America, responsible for 30% of the national production (AEN/PR, 2021).

The Guarapuava urban area is encompassed by parallels 25º 26’ 57”S and 25º 18’ 25”S and meridians 51º 35’ 23”W and 51º 22’ 36”W. The estimated population in 2021 was 183,755 habitants. The services sector is the main source of PIB generation, reaching R$ 6,105,775.17 in 2019 and representing 58% of the municipality total economy (IBGE, 2022).

2.2 Acquisition of samples

In November 2020, 56 *cerveja caseira* units were acquired at random from nine producers and were divided into 14 lots of quadrupled samples (Table 1). Production took place in October and November 2020 (personal communication).

Table 1. Characteristics and origin of the *cerveja caseira* samples acquired for analysis. Guarapuava, 2020.

Code		**Location**			**Producer/Seller**		**Date**	
Lot	**Sample**	**Color/ Type**	**Place/Sale**	**Neighborhood**	**Code**	**Name**	**Production**	**Purchase**
1	A - B - C	Light	Market	Santana	1P	T.C.	20/Oct/2020	03/Nov/2020
1	D	Light	Market	Santana	1P	T.C.	26/Oct/2020	10/Nov/2020
2	A - B – C - D	Light	Market	Vila Carli	2P	Ad.	02/Nov/2020	05/Nov/2020
3	A - B – C - D	Light	Market	Charquinho	3P	T. D.	04/Nov/2020	06/Nov/2020
4	A - B – C - D	Light	Market	Charquinho	4P	A. F.	31/Oct/2020	06/Nov/2020
5	A - B – C - D	Light	Home	Vila Bela	5P	J. M. L.	01/Nov/2020	09/Nov/2020
6	A - B – C - D	Light	Street	Saldanha/ Bus station	6P	M. B. B.	07/Nov/2020	10/Nov/2020
7	A - B – C - D	Light	Street	Santa Terezinha	7P	E. M.	06/Nov/2020	11/Nov/2020
8	A - B – C - D	Light	Grocery store	Batel	8P	Ar.	04/Nov/2020	11/Nov/2020
9	A - B – C - D	Light	Home	Bonsucesso	9P	B. L.	09/Nov/2020	11/Nov/2020
10	A - B - C	Dark	Market	Santana	1P	T.C.	20/Oct/2020	03/Nov/2020
10	D	Dark	Market	Santana	1P	T.C.	26/Oct/2020	10/Nov/2020
11	A - B – C - D	Dark	Market	Charquinho	3P	T. D.	04/Nov/2020	06/Nov/2020
12	A - B – C - D	Dark	Grocery store	Batel	8P	Ar.	04/Nov/2020	11/Nov/2020
13	A - B – C - D	Pineapple	Market	Santana	1P	T. C.	26/Oct/2020	10/Nov/2020
14	A - B – C - D	Pineapple	Home	Bonsucesso	9P	B. L.	10/Nov/2020	11/Nov/2020

As *cerveja caseira* is an unlicensed artisanal product, a previous search for selling points in

Guarapuava was made by an active in-person search and via social networks, so as to gather the largest number of producers possible.

The *cerveja caseira* units were bought in producer's markets, houses, streets, and grocery stores in Guarapuava. Nine types were named "light", three "dark", and two "pineapple"-flavored.

The units were kept in 2-L plastic bottles (PET), which did not contain any information on origin, composition, nutritional value, date of fabrication or validity (Figure 2).

Figure 2. Hop (plastic bag) and "light" and "dark" *cerveja caseira* types produced and sold in PET bottles, Guarapuava/PR.
Source: the authors

For transport, the bottles were placed in isothermal boxes containing ice, in order to maintain the characteristics of the beer and to avoid interference of external factors in quality and in the results of the microbial analyses.

2.3. Physical-chemical analyses

On 21st November 2020, a sample of each of the 14 *cerveja caseira* lots was selected and sent to a quality-control laboratory of a brewery in the State of Minas Gerais.

The samples were analyzed for alcohol content (% v/v), pH, original extract (°P), density (g/cm³), color (EBC), and apparent extract (% p/p) using an Anton Paar® beer analyzer. Bitterness (BU) and calories (kcal/100 mL) were determined using a Hach® spectrophotometer model DR5000. Manual SO_2 analyses were performed following the methodologies described in Brasil (1986) and Instituto Adolfo Lutz (2008).

2.4. Microbial analysis

During 12th November 2020 and 28th February 2021, samples from the 14 *cerveja caseira* lots were analyzed for microbial control in a microbiology laboratory of Universidade Brasil, Fernandópolis campus (State of São Paulo).

2.4.1. Sample preparation

Out each *cerveja caseira* lot (three bottles of each type/origin), 25 mL were aseptically collected and transferred to an Erlenmeyer flask containing 225 mL of sterile 0.1% peptone salt solution and homogenized. This dilution corresponded to a 1:10 (10^{-1}) proportion.

From the initial dilution, serial dilutions (10^{-2}, 10^{-3}) were performed always using the same diluent

(0.1% peptone salt solution). These dilutions were used in subsequent microbial analysis procedures performed in duplicate.

2.4.2. Total coliforms and thermotolerant coliforms

Cerveja caseira samples diluted in 0.1% peptone salt solution were tested for presumptive coliforms. 1-mL aliquots were inoculated in a series of two tubes containing Lauryl Sulfate Tryptose (LST) Broth by dilution. The tubes were incubated at 35 ºC for 24-48 hours, when the presence or absence of gas in inverted Durhan tubes was checked. The presence of gas is considered suspicious (presumptive) of the presence of coliforms (SILVA et al., 2010). After 48 hours of incubation, no gas was formed in the Durhan tubes, indicating no coliform growth and thus terminating the analytical procedure.

2.4.3. Total aerobic mesophile counts

The (10^{-1}, 10^{-2}, 10^{-3}) diluted *cerveja caseira* samples were inoculated (0.1 mL) on the surface of Standard Plate Counts (SPC). A Drigalski loop was used to spread the inoculum over the entire surface, until excess liquid was totally absorbed. After incubation at 35 ºC for 24-48 hours, the colonies were counted and the results expressed in CFU mL^{-1}.

2.4.7. Total mold and yeast counts

For total mold and yeast counts, (10^{-1}, 10^{-2}, 10^{-3}) diluted *cerveja caseira* samples were inoculated on the surface of Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) plates. After incubation at 25 °C for seven days, the colonies were counted and the results expressed in CFU mL^{-1}.

2.4.4. Coagulase-positive Staphylococcus counts

The three dilutions (10^{-1}, 10^{-2}, 10^{-3}) were spread on the surface of Baird-Parker Agar (BP) and Triptecasein Soy Agar (TSA) plates with Drigalski loops and incubated at 35 ºC for 24-48 hours. After this period, the colonies were analyzed using Gram staining under the microscope. The colonies typical of *Staphylococcus* were counted and subjected to the coagulase test.

For the coagulase test, each colony was transferred to a Brain Heart Infusion (BHI) Broth and incubated at 35 ºC for 24 hours. From the culture thus obtained, 0.2 mL were added to 0.5 mL Coagulase Plasma Rabbit with EDTA. The solution was stirred with rotation movements and avoiding shaking the tubes, so as not to interfere with clotting and then incubated at 35 ºC. The samples were checked for clots every 15 minutes for six hours. Positive reaction to the test was confirmed by the formation of clots.

2.4.5. Pseudomonas spp counts

The three dilutions (10^{-1}, 10^{-2}, 10^{-3}) were spread on the surface of plates containing Cetrimide Fucidin Cephaloridin Agar (CFC) using a Drigalski loop. The plates were incubated at 25 °C for 48 hours. To confirm the presence of *Pseudomonas*, five colonies were chosen at random from each plate. For each selected colony a Nutrient Agar (NA) plate was inoculated and incubated at 25 °C for 24 hours. Later on, a colony was selected for the oxidase and growth tests.

For the oxidase test, a filter paper disc was placed inside a Petri dish. The center of the paper was soaked with Kovacs reagent (1% aqueous solution of N,N,N,N-tetramethyl-p-phenylenediamine hydrochloride). With a sterile pick, a small amount of the culture was collected and spread over the reagent. After ten seconds, an intense blue color developed, indicating the presence of *Pseudomonas*.

For the growth test in Kligler Iron Agar (KIA) growth medium, each culture was inoculated into a KIA inclined tube with an inoculation needle by pricking and streaking on the ramp and incubated at 25 °C for 24 hours. Cultures growing on the ramp and not on the bottom of the tube were considered *Pseudomonas* spp.

2.4.6. Bacillus spp counts

0.1 mL of the three dilutions (10^{-1}, 10^{-2}, 10^{-3}) were inoculated on Mannitol Egg Yolk Polymyxin Agar (MYP) plates, followed by surface cultivation using Drigalski loops and inverted incubation at 32 °C for 24 hours.

For a quick Holbrook & Anderson test, typical colonies were selected by preparing a culture smear. The smear was covered with a 5% Malachite Green aqueous solution and hot stained for two minutes. Afterwards, the plates were cleansed with running water and covered with a Sudan Black solution for 20 minutes. Then, the plates were washed with xylene (PA) for five to ten seconds and analyzed under the microscope.

2.5. Statistical analysis

A descriptive statistical analysis of the microbial counts (determination of mean, standard deviation, and median values) was performed for each *cerveja caseira* lot. The Kruskal-Wallis test was applied to compare microbial counts. The *post-hoc* median multiple comparison test was applied for $P < 0.05$. The Principal Component Analysis multivariate tool was used to check the relationships between the cultivated microorganisms and the analyzed *cervejas caseira* types. In all tests the adopted significance level was 0.05 or 5% (ZAR, 2009). Minitab 17 (Minitab Inc.) and Statistica 12 (StatSoft) were the software used for the analysis.

3. Results and discussion

3.1. Physical-chemical analyses

Physical-chemical analyses are important parameters to set standards and characterize a beverage and its types. The results of the physical-chemical analyses of the Guarapuava *cerveja caseira* lots are presented in Table 2.

Table 2. Physical-chemical analysis of the Guarapuava *cerveja caseira* lots, 2020.

Lot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Type	Light	Light	Light	Light	Light	Light	Light	Light	Light	Dark	Dark	Dark	Pineapple	Pineapple
Color (EBC)	3.9	7.5	3.9	8.2	7.0	10.8	12.0	8.7	2.8	78.0	137.5	105.2	6.7	3.0
pH	3.50	3.78	3.83	3.60	3.73	3.79	3.65	3.65	3.93	3.44	3.20	3.20	3.25	3.56
Original extract (ºP)	8.82	14.09	13.45	14.00	12.74	11.48	10.57	11.74	10.88	13.52	14.60	11.87	11.23	9.34
Apparent extract (%p/p)	4.72	6.83	11.87	4.77	9.64	7.77	6.43	9.50	8.33	9.48	11.84	7.95	6.60	5.86
Alcohol (%v/v)	2.18	3.98	0.83	5.02	1.72	2.02	2.23	1.22	1.39	2.25	1.59	2.15	2.50	1.86
Density (g/cm³)	1.017	1.025	1.046	1.017	1.037	1.029	1.024	1.036	1.031	1.036	1.046	1.030	1.024	1.021
Bitterness (BU)	14.4	0.7	14.3	0.3	16.5	15.1	5.7	11.4	21.8	11.4	13.7	11.9	7.5	0.9
SO_2 (mg/L)	< 1	0	0	< 1	< 1	< 1	1	< 1	< 1	3	1	< 1	< 1	< 1
Calories (kcal/100 mL)	133.6	217.4	211.1	214.5	198.1	176.9	161.2	182.3	168.1	210.4	229.5	183.2	172.1	142.3

Source: Laboratory of a brewery in Minas Gerais/Brazil (2020).

In general, the artisanal drink freely sold as *cerveja caseira* in Guarapuava does not follow any standards.

Regarding the color, according to the Brazilian legislation, until 2019 a beer was considered "light" when the EBC (European Brewery Convention) value was less than 20, and "dark" when more than 20 EBC (BRASIL, 2009). This color classification is no longer applied.

In this experiment, EBC values for "light" and "pineapple" types of *cerveja caseira* vary from 2.8 to 12. The "dark" ones yield EBC between 78 and 137.5, which, according to the Beer Judge Certification Program (BJCP, 2021), they can be classifed as "black" or "opaque black".

The mean pH of several lots is 3.58, which is close to the 3.13-3.63 interval reported by Santos (2014) for the homebrewed beer produced in Ponta Grossa (State of Paraná) and 4.0 for commercial beer (ROSA; AFONSO, 2015). According to Goiana (2016), pH less than 4.5 is important to keep the beer free of pathogenic microorganisms, mainly *Clostridium botulinum*, which can cause botulism, and to avoid further contaminations, contributing to the good quality of the product. Besides contributing to the resistance to microbial changes, pH interferes in color intensity, enzymatic activity, oxidation-reduction potential, and flavor (OLIVEIRA, 2011).

The original extract of the Guarapuava *cerveja caseira* types vary from 8.82 to 14.60 ºP, with a mean value of 12.02 ºP. According to Normative Instruction 65/19, the original or primitive extract (Ep) – which is the quantity of substances dissolved in the wort and originate the beer – must always be equal or greater than 5.0 ºP (BRASIL, 2019). It is measured in degrees Plato and can be used as an analytical parameter to assess the quality of the beer production in respect to cost-benefit (AZEVEDO; SOUZA, 2021). Until 2019, according to legislation (BRASIL, 2009), beers were classified, regarding the original extract, as light (5-10.5%), common (10.5-12%), extra (12-14%), and strong (more than 14%), which qualifies the Guarapuava *cerveja caseira* types as light/common.

Considering the presence of alcohol, that is, after brewing, the apparent extract varies from 4.72 to 11.87%, with a mean value of 7.97%. Presence of alcohol correlates with the original and final apparent extract, because the greater the difference between them, the higher the alcohol content.

Alcohol content varies from 0.83% to 5.02% v/v, with a mean value of 2.21% v/v. In Guarapuava, *cerveja caseira* is consumed by people of all ages, and during the acquisition of samples, all suppliers/producers denied the inclusion of alcohol in the beverage, attesting that alcohol content results from brewing.

According to Normative Instruction 65/19, beers are classified as: I. "alcohol-free beer" – when alcohol content is equal or less than 0.5% in volume (0.5% v/v); II. "low-alcohol beer" – when alcohol content is greater than 0.5% in volume (0.5% v/v) and less or equal to 2.0% in volume (2.0% v/v), and III. "beer", when alcohol content is greater than 2.0% in volume (2.0% v/v) (BRASIL, 2019).

On average, the majority of the commercial beers contain 4.5 to 6% alcohol (ROSA; AFONSO, 2015). Santos (2014), when analyzing a homebrewed beer bought in a Ponta Grossa market and the beer produced in the Federal Technological University of the State of Paraná (UTFPR) with addition of ginger or cloves, obtained 1.5 and 1.8% v/v, respectively, that is, both are characterized as low-alcohol drink. In our experiment and according to Normative Instruction 65/19 (BRASIL, 2019), lots 1, 2, 4, 6, 7, 10, 12, and 13 are classified as "beer" and the remaining lots as "low-alcohol beer", which indicates that the type of beverage (light/dark/pineapple) is not related to alcohol content.

Regarding density, the mean value obtained was 1.02985 g/cm³, which is closely related to brewing and the alcohol content of the *cerveja caseira* types. According to Santos (2014), the density of the Ponta Grossa homebrewed beers fall between 1.02 and 1.06 g/cm³. In general, these results exceed the limits of 1.007 to 1.022 g/cm³ (SOUSA; FOGAÇA, 2019).

Bitterness is closely related to the quantity of hop used and is determined by the brewer when producing the beer. Pressed hop is used in the Guarapuava *cerveja caseira*, resulting in bitterness values varying from 0.3 to 21.8 IBU, which can be related to both the process phase when hop is added and costumers' preferences. Santos (2014) reported bitterness values of 21.35 IBU for a Ponta Grossa homebrewed beer sample. Despite bitterness is not considered in the Brazilian legislation, this quality parameter plays a fundamental role in the typical characteristics of some beer styles. Bitterness is measured in Bitterness Units (BU), and usually varies between 10 and 45 BU – the higher the value, the bitterer the beer (SILVA; FARIA, 2008). According to Collin et al. (1994), the BU range for preferred commercial beers varies from 17.5 to 25 BU.

Stable SO_2 contents between 0 and 3 mg/L were obtained for the Guarapuava *cerveja caseira* types, some of them being close to that reported by Santos (2014) of 0.14 mg/L, obtained for a homebrewed beer with addition of ginger. The limit of SO_2 contents for commercial beers is of approximately 20 mg/L. In higher concentrations, as 30 mg/L, it can cause rather unpleasant sulfurous odor and taste. Within the acceptable concentration range (< 10 mg/L), the presence of SO_2 is very important for: decreasing oxidation rate and consequently the "cardboard" taste; formation of complexes with carbonyl compounds, and antimicrobial properties (WUTTKE, 2018).

In term of calories, the values from 133.6 to 229.5 kcal/100 mL were obtained for the Guarapuava *cerveja caseira* types, which exceed those reported by Santos, of 40.08 kcal/100 mL (167.72 kj/100 mL) for a homebrewed beer sample and of 43 kcal/100 mL for commercial beers (ALMEIDA and SILVA, 2005). Higher values can be associated with greater amounts of sugar present in the Guarapuava *cerveja caseira* recipes.

3.2. Microbial analyses

All the analyzed lots lack total and thermotolerant coliforms, as recommended by Normative Instruction 65/19 (BRASIL, 2019). Even being an uninspected product, this demonstrates concern with good hygiene practices,. Nonetheless, the presence of other microorganisms, such as mesophiles, yeasts, molds, S*taphilococcus*, *Pseudomonas,* and *Bacillus* was detected.

Table 3 presents the descriptive statistics regarding total mesophiles counts for each Guarapuava *cerveja caseira* lot.

Table 3. Descriptive statistics of the total mesophiles counts for each Guarapuava *cerveja caseira* lot analyzed in this study.

Lot	Type	Mean±standard deviation	Median*		P value
1	Light	$9.1x10^5±1.3x10^6$	$3.5x10^5$	a	<0.001
2	Light	$3.7x10^4±1.1x10^4$	$4.1x10^4$	abc	
3	Light	$7.9x10^4±1.2x10^5$	$3.2x10^4$	abc	
4	Light	$2.1x10^3±7.5x10^2$	$2.4x10^3$	c	
5	Light	$2.9x10^2±1.2x10^2$	$3.3x10^2$	c	
6	Light	$2.0x10^4±7.2x10^3$	$1.7x10^4$	abc	
7	Light	$3.2x10^5±1.3x10^5$	$3.1x10^5$	ab	
8	Light	$2.6x10^4±5.1x10^2$	$2.6x10^4$	abc	
9	Light	$3.3x10^4±2.2x10^3$	$3.3x10^4$	abc	
10	Dark	$3.0x10^5±4.6x10^3$	$3.0x10^5$	ab	
11	Dark	$3.0x10^5±2.4x10^4$	$2.9x10^5$	ab	
12	Dark	$2.6x10^5±3.4x10^4$	$2.6x10^5$	ab	
13	Pineapple	$4.5x10^3±2.0x10^3$	$3.5x10^3$	bc	
14	Pineapple	$1.7x10^3±3.6x10^2$	$1.7x10^3$	c	

*Different letters in the same column indicate significantly different medians by the multiple comparison test for P <0.05.

All lots presented growth of mesophiles, with significant differences in microbial counts, when *cerveja caseira* types are compared (P <0.001). Lot 1 yielded the highest total mesophiles count, statistically differing from lots 4, 5, and 14, which yielded lower values. Lots 1, 4, and 5 correspond to the “light” *cerveja caseira* type and lot 14 to “pineapple”-flavored.

According to the *Agência Nacional de Vigilância Sanitária* legislation (Brazilian Health Regulatory Agency – ANVISA, 2010), namely *Consulta Pública* 69/2010, the maximum limit of mesophiles counts in beer is < 3000 CFU/mL. Therefore, only lots 4, 5, and 14 comply with the maximum values accepted by the legislation, the other lots being inappropriate for consumption and commercialization. However, as these beer types are artisanal, the presence of microorganisms is common, once they are not pasteurized.

According to Carneiro (2008), to obtain quality beer free of yeast residues, bacteria and coliforms, treatments such as filtration and pasteurization are necessary, as contamination with microorganisms can occur during bottling. Microorganisms can be pathogenic and harmful both for the organoleptic characteristics of the product and the consumer’s health, attesting the importance of the treatments.

Table 4 presents the descriptive statistics of yeast counts for the Guarapuava *cerveja caseira* lots. Differently from commercial beer production, any yeast present in artisanal brewing is considered wild.

Table 4. Descriptive statistics of the yeast counts for each Guarapuava *cerveja caseira* lot analyzed in this study.

Lot	Type	Mean±standard deviation	Median*		P value
1	Light	$1.6x10^2±1.3x10^1$	$1.6x10^2$	bcd	<0.001
2	Light	$1.7x10^3±2.4x10^1$	$1.7x10^3$	ab	
3	Light	$3.2x10^3±2.8x10^2$	$3.1x10^3$	a	
4	Light	$2.1x10^1±1.2x10^0$	$2.2x10^1$	d	
5	Light	$5.0x10^1±2.0x10^0$	$5.1x10^1$	cd	
6	Light	$4.6x10^2±1.1x10^2$	$4.7x10^2$	abcd	
7	Light	$4.2x10^2±1.6x10^1$	$4.1x10^2$	abcd	
8	Light	$3.2x10^2±5.7x10^1$	$3.5x10^2$	abcd	
9	Light	$8.5x10^2±8.2x10^1$	$8.7x10^2$	abc	
10	Dark	$2.2x10^3±1.9x10^2$	$2.1x10^3$	a	
11	Dark	$1.4x10^3±6.2x10^2$	$1.7x10^3$	abc	
12	Dark	$9.0x10^2±1.1x10^3$	$3.5x10^2$	abcd	
13	Pineapple	$1.6x10^2±4.2x10^1$	$1.5x10^2$	bcd	
14	Pineapple	$8.2x10^1±1.2x10^1$	$7.8x10^1$	cd	

*Different letters in the same column indicate significantly different medians by the multiple comparison test for P <0.05.

According to Table 4, all lots presented growth of yeasts, with significant count differences (P <0.001). Lots 3 and 9 (“light” types) exceeded the others, statistically differing from lots 1, 4, 5, 13, and 14, which were the least contaminated.

According to *Consulta Pública* 69/2010, the maximum limit for yeast counts is <100 CFU/mL. Thus, only lots 4 and 5 (“light”) and 14 (“pineapple”) comply with the maximum values accepted by legislation, the other lots being inappropriate for consumption and commercialization.

The contaminating yeasts can be of distinct origins and 80% of the wild yeasts belong to *Saccharomyces diastaticus*, which mainly attack, besides chopp, not-pasteurized bottled beer. As a result of this contaminated brew, the beer is characterized by turbidity, change of color, and unpleasant and phenolic flavor. *Saccharomyces cerevisiae* aerobic yeast lineages can develop together with the industrial culture and produce abnormal flavors and odors in beer, for example, diacetyl, which transfers to beer a rancid butter taste (OLIVEIRA, 2011).

Regarding mold counts, Table 5 presents the descriptive statistics for the Guarapuava *cerveja caseira* lots.

Table 5. Descriptive statistics of the mold counts for each Guarapuava *cerveja caseira* lot analyzed in this study.

Lot	Type	Mean±standard deviation	Median*		P value
1	Light	0.00±0.00	0.00	c	
2	Light	0.00±0.00	0.00	c	
3	Light	0.00±0.00	0.00	c	
4	Light	0.00±0.00	0.00	c	
5	Light	0.00±0.00	0.00	c	
6	Light	0.00±0.00	0.00	c	
7	Light	$1.0x10^1$±0.8	$1.0x10^1$	b	**<0.001**
8	Light	0.00±0.00	0.00	c	
9	Light	0.00±0.00	0.00	c	
10	Dark	0.00±0.00	0.00	c	
11	Dark	$1.0x10^1$±0.9	$1.0x10^1$	b	
12	Dark	0.00±0.00	0.00	c	
13	Pineapple	$1.6x10^1$±$7.6x10^0$	$1.3x10^1$	a	
14	Pineapple	0.00±0.00	0.00	c	

*Different letters in the same column indicate significantly different medians by the multiple comparison test for P <0.05.

The results in Table 5 indicate the presence of molds in three lots only, with significant differences in the counts (P <0.001). Lot 13 exceeded the others and presented the highest mold counts, differing from the lower values obtained for lots 7 and 11 and lots 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, and 14, where these microorganisms did not grow. There is no correlation between mold count and beer type, once lot 7 is “light”, lot 11 “dark”, and lot 13 “pineapple”.

According to ANVISA (2010), *Consulta Pública* 69/2010, the maximum limit for mold counts in beer is <100 CFU/mL. Thus, only lots 7, 11, and 13 are above the maximum values accepted by legislation, being inappropriate for consumption and commercialization.

Beer can be contaminated through airborne spores of molds of genera *Fusarium* spp*, Aspergillus* spp*, Penicillium* spp*, Rhizopus* spp, which mainly grown under low pH conditions (SOUZA; FAVERO, 2017).

Table 6 presents the descriptive statistics of *Staphylococcus aureus* counts for the Guarapuava *cerveja caseira* lots.

Table 6. Descriptive statistics of the *Staphylococcus aureus* counts for each Guarapuava *cerveja caseira* lot analyzed in this study.

Lot	Type	Mean±standard deviation	Median*		P value
1	Light	0.00±0.00	0.00	d	
2	Light	0.00±0.00	0.00	d	
3	Light	0.00±0.00	0.00	d	
4	Light	$1.2x10^1±2.2x10^0$	$1.2x10^1$	c	
5	Light	0.00±0.00	0.00	d	
6	Light	$5.4x10^1±1.0x10^1$	$5.3x10^1$	a	
7	Light	$2.9x10^1±7.6x10^0$	$3.0x10^1$	b	**<0.001**
8	Light	0.00±0.00	0.00	d	
9	Light	0.00±0.00	0.00	d	
10	Dark	0.00±0.00	0.00	d	
11	Dark	0.00±0.00	0.00	d	
12	Dark	0.00±0.00	0.00	d	
13	Pineapple	$3.5x10^1±1.3x10^1$	$3.4x10^1$	b	
14	Pineapple	$6.2x10^1±1.3x10^1$	$6.8x10^1$	a	

*Different letters in the same column indicate significantly different medians by the multiple comparison test for P <0.05.

Staphylococcus aureus grew in five lots, with significant differences in microbial counts (P <0.001) (Table 6). Lots 6 ("light") and 14 ("pineapple") yielded the highest counts*,* differing from the lower values obtained for lots 4 and 7 ("light") and 13 ("pineapple") and from uncontaminated lots 1, 2, 3, 5, 8, 9, 10, 11, and 12. Regarding type, *Staphylococcus aureus* did not grow in "dark" *cerveja caseira* types.

Staphylococcus aureus are bacteria of the *Staphylococcus* family, of the Gram-positive group, producing enterotoxins that cause food poisoning after ingestion of contaminated salted meat, potato salads, and ice cream. As these toxins do not continue to be produced within the body, the intoxication duration is considerably short, with symptoms starting four hours after ingestion and lasting for 24 hours at most. Contrarily to other intoxications, food contamination by *Staphylococcus* usually occurs by hand contact with infected people, who very often do not know about the disease, because they are asymptomatic (MURRAY et al., 1998).

Staphylococcus aureus bacteria are frequently lodged on the skin and in the nose, being transmitted via wounds in the hands or other purulent lesions, inhalation of contaminated droplets eliminated by coughing or sneezing and by infected objects or food, which indicates poor hygiene practices (FDA, 2012).

Table 7 presents the descriptive statistics of the *Pseudomonas* counts for the Guarapuava *cerveja caseira* lots.

Table 7. Descriptive statistics of the *Pseudomonas* counts for each Guarapuava *cerveja caseira* lot analyzed in this study.

Lot	Type	Mean±standard deviation	Median*		P value
1	Light	0.00±0.00	0.00	c	
2	Light	$1.1x10^1 ± 4.0x10^0$	$1.0x10^1$	b	
3	Light	0.00±0.00	0.00	c	
4	Light	$1.1x10^1 ± 1.5x10^0$	$1.1x10^1$	b	
5	Light	0.00±0.00	0.00	c	
6	Light	0.00±0.00	0.00	c	
7	Light	0.00±0.00	0.00	c	**<0.001**
8	Light	0.00±0.00	0.00	c	
9	Light	0.00±0.00	0.00	c	
10	Dark	0.00±0.00	0.00	c	
11	Dark	0.00±0.00	0.00	c	
12	Dark	$5.7x10^2 ± 1.3x10^3$	$4.0x10^1$	a	
13	Pineapple	$3.7x10^1 ± 2.2x10^1$	$4.0x10^1$	a	
14	Pineapple	0.00±0.00	0.00	c	

*Different letters in the same column indicate significantly different medians by the multiple comparison test for P <0.05.

The results listed in Table 7 reveal the presence of *Pseudomonas* in four lots, with significant differences in microbial counts (P <0.001). Lots 12 and 13 yielded the highest counts, differing from the lower values obtained for lots 2 and 4 and the other uncontaminated lots. No correlation exist between *Pseudomonas* counts and beer type, once lots 2 and 4 are “light”, lot 12 is “dark”, and lot 13 is “pineapple”-flavored.

The genus *Pseudomonas* spp is composed of bacteria in the form of Gram-negative bacilli, which are non-fermenting, aerobic, mobile, opportunistic and ubiquitous, and can be isolated from soil, water, plants, food and surfaces (OCHOA et al., 2013).

In food industry, the importance of *Pseudomonas* is related to the deterioration and shorter shelf life of certain types of food, such as processed milk, raw vegetables, pastry products, and unpasteurized juices. These microorganisms can cause bad odors, viscidity, and bad taste (ABDUL-MUTALIB et al., 2019).

Table 8 presents the descriptive statistics of the *Bacillus* counts for the Guarapuava *cerveja caseira* lots.

Table 8. Descriptive statistics of the *Bacillus* counts for each Guarapuava *cerveja caseira* lot analyzed in this study.

Lot	Type	Mean±standard deviation	Median*		P value
1	Light	0.00±0.00	0.00	b	<0.001
2	Light	0.00±0.00	0.00	b	
3	Light	0.00±0.00	0.00	b	
4	Light	0.00±0.00	0.00	b	
5	Light	$2.3x10^1±5.1x10^0$	$2.0x10^1$	a	
6	Light	0.00±0.00	0.00	b	
7	Light	0.00±0.00	0.00	b	
8	Light	0.00±0.00	0.00	b	
9	Light	0.00±0.00	0.00	b	
10	Dark	0.00±0.00	0.00	b	
11	Dark	$1.6x10^1±5.1x10^0$	$2.0x10^1$	a	
12	Dark	$2.8x10^1±3.0x10^1$	$2.0x10^1$	a	
13	Pineapple	0.00±0.00	0.00	b	
14	Pineapple	0.00±0.00	0.00	b	

*Different letters in the same column indicate significantly different medians by the multiple comparison test for P <0.05.

The results in Table 8 indicate the presence of *Bacillus* in three lots, with significant differences in microbial counts (P <0.001). Lots 5 (“light”), 11 and 12 (“dark”) yielded the highest counts, differing from the other uncontaminated ones. Thus, regarding the beer type, *Bacillus* was not detected in the “pineapple”-flavored *cerveja caseira* type.

The genus *Bacillus* is a Gram-positive aerobic bacteria, commonly found in breweries, being a potential contamination agent, once they are present in dust, surfaces, equipment, and brewing adjunct like sucrose, surviving the boiling of wort, only inhibited by low pH and the presence of hop. Unpasteurized beers are more potentially contaminated during bottling (SILVA, 2019).

In brewing, other Gram-positive microorganism genera are described: *Lactobacillus, Acetobacter, Clostridium, Leuconostoc, Pediococcus,* and *Micrococcus*, which produce considerable quantities of organic acids (butyric, acetic, formic, lactic) (ANDRIETTA; STECKELBERG; ANDRIETTA, 2006).

3.2.1. Multivariate analysis of microbial parameters

Multivariate analysis of identification and microorganism count data was performed using the Principal Component Analysis tool. This tool consists of restructuring the data in a set of data derived from the original ones, grouping them in principal components that explain a percentage of the total variation. The better the explanation of the variation from the original data by this derived data set, the more accurate the analysis is.

Figure 3 shows the behavior of the assessed microorganisms and the *cerveja caseira* lots in two 2-D graphs. Component 1 responds for 28.01% and component 2 for 27.19% of the total variation of the data.

When analyzed together, both components respond for 55.20% of such variation.

Yeasts present strong positive correlation with component 1, whereas *Staphylococcus aureus* present strong negative correlation with such component. *Pseudomonas* and *Bacillus* are positively correlated with component 2. Total mesophiles and molds did not significantly contribute to the analysis (FIGURE 3A).

Analyzing the lots, it is possible to assume that lot 12 yielded the highest *Pseudomonas* and *Bacillus* counts, for being superposed to its respective autovectors. Lots 6, 13, and 14 yielded the highest *Staphylococcus aureus* counts in quadrant 3, and lot 3, the highest yeast counts (FIGURE 3B).

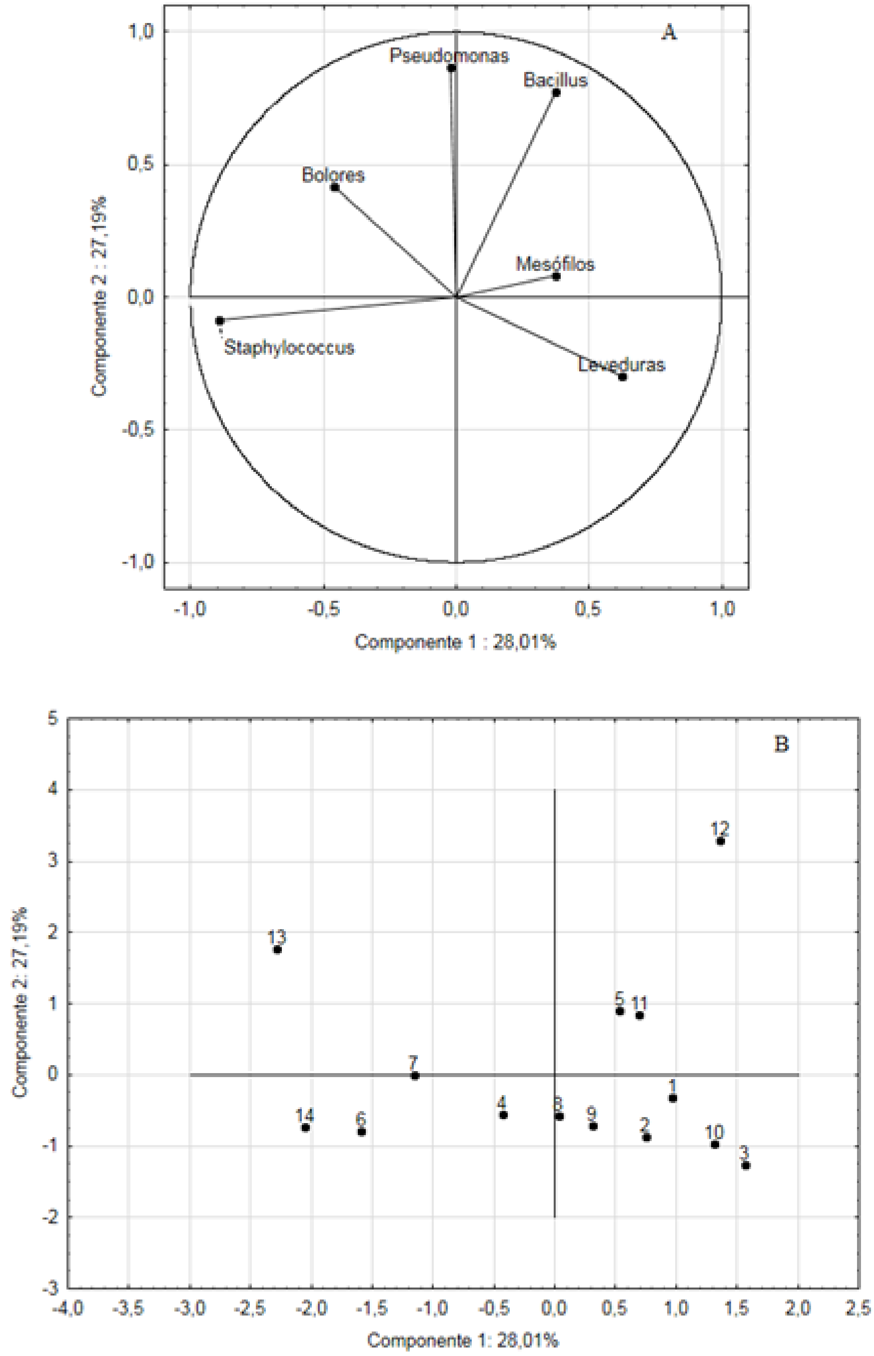

Figure 3. 2-D projection of microorganisms and Guarapuava *cerveja caseira* types generated by Principal Component Analysis (PC1 and PC2).

Figure 4 shows the behavior of the assessed microorganisms and the *cerveja caseira* lots in two 2-D graphs. Component 1 responds for 28.01% and component 3 for 17.43% of total variation of the data. When analyzed together, both components respond for 45.07% of such variation.

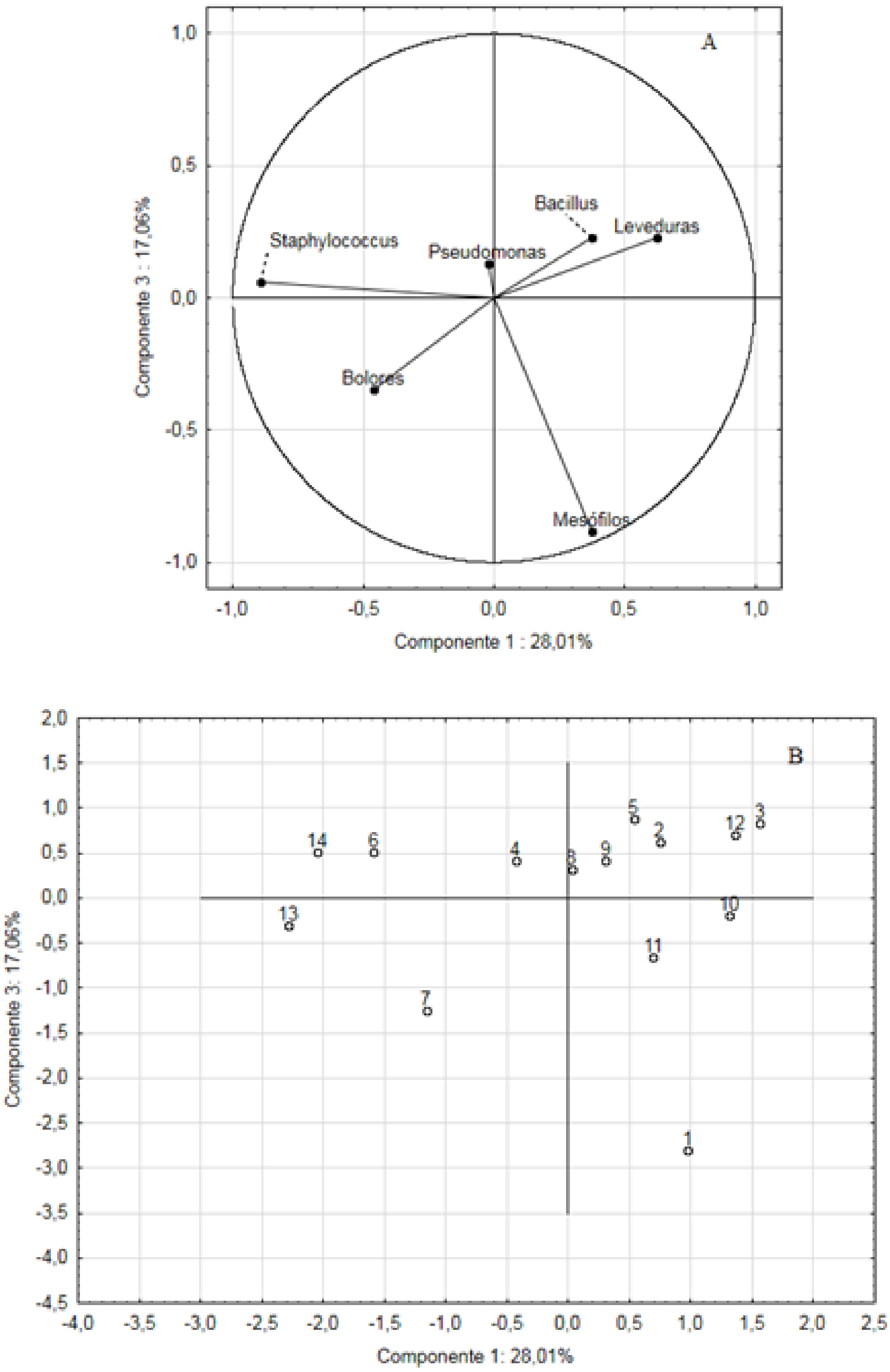

Figure 4. 2-D projection of microorganisms and Guarapuava *cerveja caseira* types generated by Principal Component Analysis (PC1 and PC3).

Yeasts and *Staphylococcus aureus* present strong positive and negative correlations respectively with component 1. Total mesophiles present strong negative correlation with component 3. Combining components 1 and 3, the contributions of molds, *Pseudomonas*, and *Bacillus* are not significant (FIGURE 4A).

Analyzing the lots according to their superpositions to their respective autovectors, it is possible to suppose that lot 12 yielded the highest *Pseudomonas* and *Bacillus* counts in their respective quadrants; lots 13 and 14 yielded the highest molds and *Staphylococcus aureus* counts, respectively in quadrants 1 and 2, and lot 1 yielded the highest total mesophiles count in quadrant 4 (FIGURE 4B).

Thus, for the multivariate analysis in general, lot 1 yielded the highest total mesophiles count; lots 6, 13, and 14 the highest *Staphylococcus aureus* count; lot 12 the highest *Bacillus* and *Pseudomonas* counts, and lot 3 the highest yeasts count. The contribution of molds was not significant.

4. CONCLUSION

A few studies exist regarding the historical/cultural importance and nutritional properties of homebrewed beer. Great variations in physical-chemical characteristics, mainly color, original and apparent extracts, alcohol content, and bitterness, can be attributed to the lack of standards in production.

Regarding the microbial analyses of the Guarapuava *cerveja caseira*, all lots presented pathogenic agents, mainly mesophiles, yeasts, and *Staphylococcus aureus*, but lack of total and thermotolerant coliforms, important contamination indicators. According to the Brazilian legislation, only lot 4 could be commercialized, despite the high alcohol content of 2.5% v/v, which characterizes it as an alcoholic drink, inappropriate for children and adolescents.

Cerveja caseira recipes have passed on from generation to generation, have been adapted to the climate and products available in Brazil, and are part of the cultural heritage of the western region of Paraná that must be preserved.

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