Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.102022



# A newer source of microorganism to produce Catharina Sour beers

Grace GHESTI[1]*, Igor CARVALHO[1], Talita CARMO[1], Paulo A. Z. SUAREZ[1]

**Abstract**

Brazil's beer industry has experienced a significant growth over the recent years, driving professionals to seek brewing beer focused on quality aspects. As a result of this movement, the consumer attention to products that highlight the national or local identity has rocketed in recent times, especially Brazilian types of beers, as Catharina Sour. Catharina Sour beer was produced using the traditional method to acidify the wort by lactic fermentation and using a bee pollen, as a newer source of microorganism to produce sour beers. For that, mango and passion fruit were used, including barley and wheat malt, yeast, and hop. Some physicochemical analyses were made according to EBC (European Brewery Convention) guidelines, to raise information about the process and final product. There were close correlations between the physic-chemical, sensory and liquid chromatography analysis, showing that the presence of pollen, and consequently the presence of yeast, acetic acid bacteria and fructophilic lactic acid bacteria (FLABs), can bring different aromas to Catharina Sour beers, showing an excellent potential to use this newer source of microorganisms to produce sour beers.

**Keywords:** Catharina Sour; brewing process; bee pollen; fructophilic lactic acid bacteria; yeast.

**Practical Application:** A newer source to produce Catharina Sour beers based on bee-products.

## 1 Introduction

Beer production worldwide was growing until 2012 when it reached its peak with about 200 billion liters annually, since then the produced volume has been stable (Muller et al., 2021). Meanwhile, Brazilian beer market has been gradually increasing, mostly due to the craft beer industry, which raised 23% annually (Lima et al., 2017). Nowadays, the Brazilian beer industry moves around US$ 35 billion a year, accounting for 1.7% of Gross National Income (GNI) and employs 2.7 million people. According to the Ministry of Agriculture, there are 1,383 registered breweries in the country. Even with the pandemic, the number of breweries grew by 14.1%. Most of them are small and medium-sized establishments, which produce special beers (Costa et al., 2021). Despite the increasing number of craft breweries registered in the country, artisanal brands account only for 1% of national production. Indeed, according to CervBrasil, in 2021 59% of beer were produced by the 10 best-selling brands in the country. However, this production concentration was higher in 2014 (70%) and is decreasing due to the growth of premium brands and special beers.

Currently, beer styles are validated through the guide called BJCP (Beer Judge Certification Program), an organization that aims to standardize and adjust the guidelines for matching beer styles (Beer Judge Certification Program, 2021). To date, there are 154 beer styles catalogued, ranging from well-known beer styles such as Pilsen to beers with the addition of spices, woods, honey, fruits or even roots. Within this context, there is the Catharina Sour style, genuinely Brazilian and officially recognized by the BJCP in the year 2021 (Silva et al., 2022). Catharina Sour is characterized as a refreshing sour beer with the addition of fruits and a clean lactic acidity taste. Indeed, this style has a strong presence of acidity and ABV (Alcohol by volume) between 4% and 5.5%. The low alcohol content, light body, high carbonation, and low bitterness make the fresh fruit stand out (Beer Judge Certification Program, 2021).

It is a barley/wheat beer prepared after three different fermenting steps. After a first boiling, a lactic fermentation is carried out by the addition of *Lactobacillus*. Craft breweries usually uses commercial probiotic beverages containing live *Lactobacillus*. When the desired acidity is achieved, the wort is boiled again accomplished by the addition of hop. Then, an alcoholic fermentation take place after inoculating ale yeast. When achieving approximately 80% of the desired gravity drop fruit pulp is added and the wort is left fermenting to the desired gravity. The possibilities of fruits can be listed: raspberry, grape, pineapple, strawberry, peach as well as pitaya and passion fruit (Silva et al., 2022).

It the last decades, different works showed that stingless bees (Hymenoptera: Apidae: Meliponini), which are a group of eusocial insects inhabiting tropical and subtropical regions, rely on bacteria and yeast fermentation to preserve honey and transform pollen in stored food (bee bread) (Paula et al., 2021). The major bacterial associated with stingless bees are *Lactobacillus*, *Bacillus*, *Streptomyces*, *Clostridium*, *Staphylococcus*, *Streptococcus*, *Enterobacter*, *Ralstonia*, *Pantoea*, *Pseudomonas*, *Fructobacillus*, *Lysinibacillus*, and *Neisseria*. On the other hand, the yeast species most frequently isolated from stingless bees belong to

*Received 07 Oct., 2022*
*Accepted 12 Dec., 2022*
[1] *Universidade de Brasília – UnB, Brasília, DF, Brasil*
*Corresponding author: ghesti.grace@gmail.com; grace@unb.br

the genus *Starmerella*. "Bee-derived" products are a rich source of fructophilic lactic acid bacteria (FLABs), and there are some interesting observations about FLABs: (a) they have already been detected in the digestive tracts of pollinators, such as insects, bees and ants; (b) osmotolerance to higher concentrations of sugar (45 to 50% weight/volume); (c) preference for the consumption of fructose than glucose; (d) they have antibacterial activity, are more apt to use more complex carbohydrates (Maeno et al., 2021; Gustaw et al., 2018).

Therefore, the present study aims to produce and characterize a Catharina Sour beer but evaluating the use of *Frieseomelitta varia*, a Brazilian stingless bee from the Cerrado bioma, bee bread as a new source of lactobacillus for Catharina Sour beer style preparation. In this study, the microbiota in the bee bread was studied and a Catharina Sour beer was prepared using this microbiota for lactic fermentation and a mixture mango and passion fruit pulps. The physical-chemical, biological and sensorial tests was carried out to map and scientifically discuss the intrinsic aspects related to beer.

## 2 Materials and methods

### *2.1 Materials*

For the beer production, 3 kg of Pilsen malt (Agraria, Brazil) and 3 kg of wheat malt (Agraria, Brazil), 15 g of Magnum hop as pellet (Hopsteiner, Germany), 15 g of ale yeast US-06 (Fermentis, France), 80 g of commercial lactobacillus beverage (Yakult) or 15 g *Frieseomelitta varia* bee bread, 1 kg of passion fruit pulp and 1 kg of mango fruit pulp (Brasspolpa, Brazil).

### *2.2 Brewing process and beer analyses*

Sample beers were brewed 50% Pilsen malt and 50% wheat malt. For the mashing curve, it was made rest of 10 min at 52 °C, 20 min at 63 °C, 20 min at 71 °C and 10 min at 78 °C. It was used 21 L of primary water and 14 L of secondary water. The wort boiled for 10 min, cooled down to 45 °C and after that the *Lactobaccilus* (LCS) or pollen (CSP) was added. Then, the wort was kept at 45 °C until achieved pH 3.5 and, in sequence, boiled again for 70 min, being added 15 g of magnum hop remaining 30 min. When finished, the wort was cooled down to room temperature and was added the ale yeast Fermentis US06 - 1.500.000 cells/mL ºPlato (Fermentis, France) and kept fermenting at room temperature. When the apparent gravity achieved 1.010, mango and passion fruit pulps were added. The wort was fermented for 168 h, bottled with 5 mL of primer, and maturated at room temperature for other 168 h. pH and gravity were measured according to EBC 8.17 and EBC 8.2.2 standard methods, respectively. Beer physicochemical characteristics such as alcohol content and fermentability were measured with PBA-B M-AntonPaar equipped with AlcolyzerPlus Beer and density detector DMA 5000 M (AntonPaar, Austria).

Fermentable carbohydrates and organic acids were measured by EBC 8.17 with an Phenomenex Rezex ROA-Organic Acid H+ column (300 × 7.8 mm) and UV detector SPD-20A for organic acids and refraction index detector RID-10A for carbohydrates and alcohols. The fermentation byproducts were analyzed using a high-performance liquid chromatography equipment (HPLC; Shimadzu, Kyoto, Japan), with isocratic mobile phase elution ($H_2SO_4$ 0.5 mM, 0.6 mL/min). The analytical parameters of the chromatographic analyses were determined qualitatively with 95% precision.

Pollen was divided into three samples: (A) used for total RNA extraction; (B) for selection of bacteria using Luria Bertani (LB) culture medium; and (C) for selection of possible yeasts with YPD medium (yeast extract 1%, peptone 2% and dextrose 2%). Samples (B) and (C) were incubated for 16 h at 30 °C and 200 rpm before the extraction process. RNA extraction was performed with TRIzol (Thermo Fisher Scietific) and RNeasy Plus Mini Kit (QIAGEN) reagents. The quantification of total RNA was made according to the recommendations of QUBIT, a fluorimetric specific method for RNA, with greater precision in relation to the traditional spectrophotometric method, because it uses specific dyes for RNA, DNA, and proteins. The microbiota analysis was performed with Bioanalyser Agilent 2100 (Agilent Technologies, standard protocol for RNA), to make an RNA similarity comparison with the world database and to analyze its integrity, capillary electrophoresis analysis of the samples was performed through this system. The quality of RNA was verified by the RNA Integrity Number - RIN.

Sensorial analysis results were divided into aromas and flavor for each sample. The file had a 1-5 grading system, where 1 was the least and 5 the highest sensorial perception. The average perception for each characteristic was calculated and expressed as a spider graph for visual representation.

## 3 Results and discussion

### *3.1 Frieseomelitta varia pollen microbiota analysis*

Bees collect pollen and nectar according to the availability of botanical resources within their foraging ranges which are affected by environmental and seasonal factors (Selvaraju et al., 2019). Bee pollen is a mixture of plant pollen pellet with nectar, honeybee secretions and microorganisms. The nutritional components in bee pollen include carbohydrates, proteins, lipids, vitamins, minerals, polyphenols, and a small percentage of other components. Previous studies demonstrated that bee pollen exhibit antioxidant, antibacterial, anti-inflammatory, anticarcinogenic, and antiallergic properties (Li et al., 2018).

The Bioanalyzer allowed a qualitative and quantitative analysis of each RNA sample. The parameter measured was the RNA Integrity Number – RIN, the most important for sequencing. The amount of RNA present in the samples is of high importance for the sequencing, as only samples with an RNA concentration equal to or greater than 40 ng/μL are viable. The concentration of RNA obtained in the evaluated samples was 600 ng/μL for sample A (pollen), 420 ng/μL for sample B, and 180 ng/μL for sample C. The RIN value was calculated by an algorithm that analyzes the results obtained and assigns a score to the samples, which varies from 1 to 10. For prokaryote and eukaryote samples, the RIN value equal to or greater than 7 is considered optimal and the samples are approved for further analysis. Only 2 samples: (A) total RNA extraction from pollen samples and (B) selection of bacteria using Luria Bertani (LB) culture medium had RIN above 7. The literature presents RNA

quality methods for gene expression studies, showing that the quality and integrity of samples is fundamental for the success of next-generation sequencing. Preliminary analyzes of Sanger sequencing indicated the presence of fructophilic lactic acid bacteria (FLAB), especially *Fructobacillus* and *Lactobacillus*.

### *3.2 Beer preparation and caracterization*

Two different Catharina Sour style beers were prepared using similar procedure but changing the microorganisms used for lactic fermentation: one using a commercial beverage containing live *Lactobaccilus* (LCS) and other using *Frieseomelitta varia* pollen (CSP). For LCS, after the first boiling (10 min) the pH was 5.4 and density of 1.030 (7.55 ºP). At that moment, 80 g of the commercial beverage was added. After 27 h, the pH dropped to 3.5, and the second boiling process (70 min) was started for protein coagulation and *lactobacilli* inactivation. So, the wort was cooled down to room temperature. At this moment the measured pH was 3.5 and density was 1.030 (7.55 ºP). After left fermenting for 72 h at 20 °C, the density dropped to 1.010 (2.56 ºP) and passion fruit and mango pulps were added. After 72 h of fermentation, the density was 1.005 (1.28 ºP) and the pH 3.4. At this moment the beer was bottled with 5 mL of 50 mass% sugar/water prime. For CSP, a similar procedure was carried out and the only difference was the use of pollen instead of the commercial probiotic beverage for the lactic fermentation. It is worth to mention that similar behaviors were observed: after the first boiling process the pH was 5.9 and density was 1.030 (7.55 ºP); after 24 h the measured pH was 3.5; and after total fermentation, the density was 1.002 (0.51 ºP) and pH of 3.4.

The analysis of final beer samples and their physicochemical parameters are displayed in Table 1. It is important to highlight that those analysis were conducted after maturation process (final product evaluation). Despite having similar mash properties, final beer differed significantly in all physicochemical parameters analyzed unless the specific gravity which was the same. LCS had a higher alcohol content and lower acid index than CSP, probably due to a more intense lactic fermentation when using pollen microbiota. LCS had also a higher real extract and calories, which is expected because of its higher alcohol content. Add to that, the value of color was 10 EBC for both and the style recommends 7 EBC. But the color of the final beer has the contribution of the color fruit. The bitterness, for both samples, were 5 BU and are within the expected for the style. The overall physicochemical properties of CSP suggests a beer with lower acidity index, calories, and alcohol content taste less apparent and with greater body than LCS reference beer.

### *3.3 Chromatography analysis – byproducts compound formation*

HPLC analysis identified sugars, acetate, and acids for LCS and CSP beers, as listed in Table 2. The remaining sugars content gave a perspective on the metabolism of yeast and bacteria during fermentation and the identification of some compounds by HPLC suggests about the behavior of the pollen microbiota in relation to wort. The taste of citric and fruits in CSP was confirmed by the presence of citric acid and isovaleric acid in higher concentration than LCS. Phenylethyl acetate was identified, and this production is strictly related to the nitrogen catabolic pathway, suggesting no yeast stress as they are repressed in such conditions. This molecule has a fruity or floral aroma (Holt et al., 2019) and it was identified by the sensorial panel.

**Table 1**. Physicochemical properties of Catharina Sour prepared using *Lactobacillus* from a commercial probiotic beverage (LCS) and from *Frieseomelitta varia* pollen (CSP) beers.

| Parameter | LCS | CSP |
|---|---|---|
| Specific gravity (g/mL) | 1.005 ± 0.10 | 1.005 ± 0.10 |
| Real degree of Fermentation (%) | 67.76 ± 0.10 | 66.60 ± 0.34 |
| Alcohol (% v/v) | 4.24 ± 0.00 | 3.83 ± 0.08 |
| Real extract (% m/m) | 3.28 ± 0.05 | 3.12 ± 0.15 |
| Calories (kJ/100 mL) | 146.5 ± 3.00 | 134.52 ±2.00 |
| Apparent degree fermentation (%) | 82.52 ± 0.20 | 81.20 ± 0.20 |
| Apparent extract (% m/m) | 1.72 ± 0.01 | 1.70 ± 0.00 |
| Remain sugar (ºBailling) | 1.70 ± 0.00 | 1.72 ± 0.02 |
| pH | 3.5 | 3.4 |
| Acidity index (% lactic acid) | 8.6 ± 0.1 | 9.7 ± 0.1 |

**Table 2**. Chemical compounds identified by HPLC in LCS and CSP.

| Chemical compound | | Concentration (g/L) | |
|---|---|---|---|
| | | LCS | CSP |
| *Alcohols* | Ethanol | 3.50 | 3.95 |
| *Esters* | Phenylethyl acetate | 0.34 | 0.45 |
| *Acids* | Acetic | 1.87 | 1.22 |
| | Succinic | 7.34 | 6.21 |
| | Citric | 3.02 | 3.80 |
| | Lactic | 9.69 | 9.99 |
| | Isovaleric | 4.52 | 4.82 |
| *Sugars* | Glucose | n/a | n/a |
| | Sucrose | 0.94 | 0.92 |
| | Maltose | 0.55 | 0.38 |
| | Lactose | 2.02 | 1.96 |

### *3.4 Sensorial analysis*

The sensorial panel found some aroma in either beer (Figure 1), with sweet fruits, citric, valeric acid and green fruits have being prominent ones, 2 (LCS) and 4 (CSP), on a 5-grading system. CSP had more sweet and green fruits aromas probably due to the pollen added. The taste of citric and fruits, also an equilibrium of composition were detected stronger than in LCS, that presented more valeric acid, who harmed the sensory analysis. The visual aspects were similar presenting same turbidity and a paler color as expected from them.

For the LCS sample, the sensorial panel identified a very pronounced isovaleric acid off-flavor, which made it difficult to assess the other attributes of the beer. For this beer style, it is a very common off flavor, but unpleasant to the palate. Despite LCS has lower isovaleric acid determined by HPLC, probably it is more apparent than in CSP because of some contribution of the pollen. Indeed, the general impression is that the CSP sample was more balanced in all requirements, presenting a higher score in general aspect, according to Figure 2.

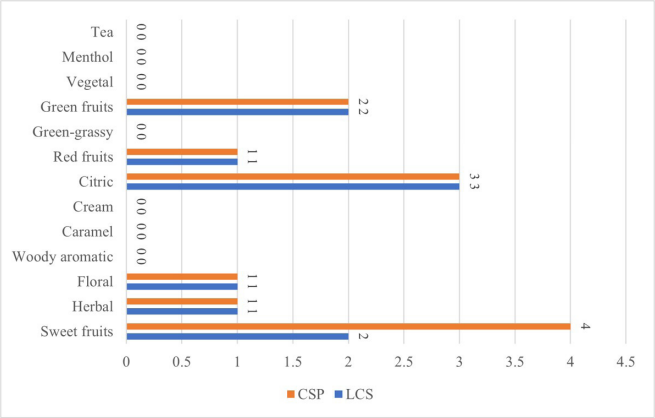


**Figure 1**. Sensory Analysis with description of flavors.

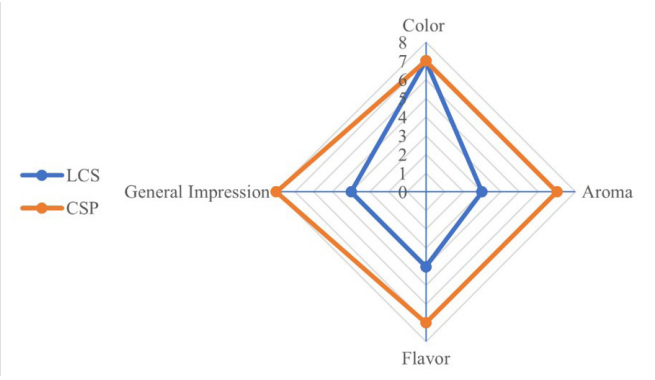


**Figure 2**. Aroma (A) and taste (B) profiles of CSP (red) and LCS (blue).

In the Brazilian market, national malt, both wheat and barley, and hops are already being commercialized, adding value to the product and unique characteristics for the beers. So, the preparation of beer with the terroir of the region and the use of bee pollen can add a lot of particularities, since they differ based on a variety of factors, including botanical origins, bee species, and geographic origins (Li et al., 2018). On the other hand, the use of bee pollen as a new source of microorganisms in the beverage and food industry may have a high potential. Indeed, fermentation is one of the oldest and most economical methods to produce preserved food. In addition, fermented foods and beverages, containing lactic acid bacteria, are considered as probiotic products and they are of great significance for human health (Iskakova et al., 2019) and stingless bee pollen may be an excellent source.

## 4 Conclusions

There is a close correlation between the sensory analysis and the liquid chromatography analysis, showing that the presence of pollen, and consequently the presence of yeast, acetic acid bacteria and fructophilic lactic acid bacteria (FLABs), can bring different aromas to Catharina Sour beers.

In other words, bee pollen can be used as a source of acidification for sour beers, allowing a multitude of possible combinations for the creation of new beers and even the creation of new beer styles.

## Acknowledgements

The authors appreciate the financial support of the National Council for Scientific and Technological Development (CNPq), the Coordination of Superior Level Staff Improvement (CAPES) and the Federal District Research Support Foundation (FAPDF), also would like to thank Agrária Sempre Viva and Maltes Catarinenses for providing the buckwheat samples for this study, Drinks Counterfeiting Laboratory of the Federal District Civil Police (PC-DF) for the utilization of equipment for physicochemical beer analysis, Molecular Biology Laboratory from the University of Brasília (IB/UnB) and also the Institute of Chemistry (IQ/UnB) for providing laboratory installations for analysis and experimental data acquisition.

## References

Beer Judge Certification Program – BJCP. (2021). *Beer style guide*. Retrieved from https://www.bjcp.org/ bjcp-style-guidelines/

Costa, S. A., Evaristo, R. B. W., Silva, M. L., & Ghesti, G. F. Prospecção tecnológica e científica de bioprocessos de conversão do bagaço de malte com ênfase em compostagem e biodigestão. *Revista Virtual da Química*. In press.

Gustaw, K., Michalak, M., Polak-Berecka, M., & Waśko, A. (2018). Isolation and characterization of a new fructophilic *Lactobacillus plantarum* FPL strain from honeydew. *Annals of Microbiology*, 68(7), 459-470. http://dx.doi.org/10.1007/s13213-018-1350-2. PMid:29983672.

Holt, S., Miks, M. H., de Carvalho, B. T., Foulquié-Moreno, M. R., & Thevelein, J. M. (2019). The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *FEMS Microbiology Reviews*, 43(3), 193-222. http://dx.doi.org/10.1093/femsre/fuy041. PMid:30445501.

Iskakova, J., Smanalieva, J., & Methner, F. J. (2019). Investigation of changes in rheological properties during processing of fermented cereal beverages. *Journal of Food Science and Technology*, 56(9), 3980-3987. http://dx.doi.org/10.1007/s13197-019-03865-9. PMid:31477969.

Li, Q. Q., Wang, K., Marcucci, M. C., Sawaya, A. C. H. F., Hu, L., Xue, X. F., Wu, L. M., & Hu, F. L. (2018). Nutrient-rich bee pollen: a treasure trove of active natural metabolites. *Journal of Functional Foods*, 49, 472-484. http://dx.doi.org/10.1016/j.jff.2018.09.008.

Lima, L. A., Fernandes, T. L., Tenório, L. X. S., Silva, M. L., Evaristo, R. B. W., Ghesti, G. F., & Martin, A. R. (2017). Sinopse do cenário cervejeiro: o advento da produção e o mercado na região centro oeste. *Cadernos de Prospecção*, 10(4), 650-664. http://dx.doi.org/10.9771/cp.v10i4.23041.

Maeno, S., Nishimura, H., Tanizawa, Y., Dicks, L., Arita, M., & Endo, A. (2021). Unique niche-specific adaptation of fructophilic lactic acid bacteria and proposal of three *Apilactobacillus* species as novel members of the group. *BMC Microbiology*, 21(1), 41. http://dx.doi.org/10.1186/s12866-021-02101-9. PMid:33563209.

Muller, C. V., Guimarães, B. P., & Ghesti, G. F. (2021). O controle oficial de uso de adjuntos em cerveja no Brasil. *Revista Processos Químicos*, 15(29), 69-81. http://dx.doi.org/10.19142/rpq.v15i29.591.

Paula, G. T., Menezes, C., Pupo, M. T., & Rosa, C. A. (2021). Stingless bees and microbial interactions. *Current Opinion in Insect Science*, 44, 41-47. http://dx.doi.org/10.1016/j.cois.2020.11.006. PMid:33271364.

Selvaraju, K., Vikram, P., Soon, J. M., Krishnan, K. T., & Mohammed, A. (2019). Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. *Journal of Food Science and Technology*, 56(5), 2508-2521. http://dx.doi.org/10.1007/s13197-019-03728-3. PMid:31168133.

Silva, C. H. G., Oliveira, L. L., Westphal, T. T., Rocha, A. F., Iglikovski, S. M., & Ceola, D. (2022). Caracterização da cerveja Catharina Sour produzida com insumos catarinenses. *Brazilian Journal of Development*, 8(5), 38180-38198. http://dx.doi.org/10.34117/bjdv8n5-359.