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# Unlocking the microbial potential of *Frieseomelitta varia* pollen for innovative probiotic mead development

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**Abstract**

This study explores an innovative approach to mead production by incorporating *Frieseomelitta varia* bee pollen as a microbial starter to promote probiotic fermentation. Mead was produced using 5 kg of honey (82° Brix, pH 4.5), 26 L of filtered water, 20 g of pollen for lactic fermentation, and 10 g of *Saccharomyces cerevisiae* (M05 Mead Yeast) for alcoholic fermentation. A total of six sanitized fermentative reactors were used with varied pollen concentrations (1–20 g/2 L). The process included wort preparation (boiling, cooling, and mixing), 24 days of fermentation, 2 months of maturation, and final bottling. Samples were analyzed using standardized methods for alcohol content, pH, original and final gravity, color (ultraviolet–visible [UV–vis], 430 nm), and volatile profiles (gas chromatography-mass spectrometry [GC-MS]). Sensory evaluation assessed aroma and flavor on a 1–5 scale. The results showed that *F. varia* pollen supports microbial activity, yielding a mead with probiotic counts above $10^6$ colony-forming unit (CFU) $mL^{-1}$. The addition of pollen enhanced physicochemical characteristics and sensory properties, producing a probiotic mead with potential health benefits. This approach not only improves product quality but also aligns with growing market interest in functional and fermented beverages.

**Keywords:** mead; probiotic beverage; pollen; fermented drink; probiotic mead; honey.

**Practical Application:** A direct and practical application of this study is the development of a new category of functional mead beverages, specifically tailored for health-conscious consumers seeking both innovative flavors and probiotic benefits.

## 1 INTRODUCTION

Mead has been a beverage produced since ancient times in various regions of the world, especially in the European continent (Schwarz et al., 2020), and has been gaining ground in the market due to its bioactive potential, attributed to honey and other ingredients used in its making (Kawa-Rygielska et al., 2019; Mendes-Ferreira et al., 2010; Silva et al., 2020). Currently, the global honey production has reached the mark of 1.4 million tons, with China, the United States, and Argentina leading the production. Annually, the honey production chain generates over R$ 950 million, and in 2022, it surpassed the production record with 61,000 tons (A.B.E.L.H.A., 2024). Compared to the previous year, the production increased by 9.5%, driven by the growing demand for honey and its derivatives, such as propolis and royal jelly (Gebrim, 2011).

The ingredients used for preparing the beverage have a significant impact on the fermentation process and can modify the bioactive potential of mead (Amorim et al., 2018; Kawa-Rygielska et al., 2019). Studies on the characteristics of meads with added herbs and fruits have been published recently (Cavanholi et al., 2021; Kawa-Rygielska et al., 2019; Romano et al., 2021). In this regard, possibilities arise for the addition of elements capable of altering the sensory characteristics of mead and even enabling a probiotic potential, as has been done with beers previously (Ghesti et al., 2023).

Stingless bees, known as meliponines, play a crucial role in pollinating various plants, relying mainly on carbohydrates and proteins obtained from pollen and nectar as their diet

(Santos et al., 2023). Pollen, also referred to as bee bread, serves as a rich source of proteins, vitamins, and essential minerals crucial for bee growth, preserved intact by the presence of lactic acid bacteria (LAB) (Anderson et al., 2014), offering a variety of medicinal benefits such as antibacterial properties, tumor growth inhibition, antioxidant effects, and protection of the nervous system and liver (Abouda et al., 2011).

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The transformation of elements produced by bees through fermentation is closely linked to the presence of microorganisms in the colony. Fermentation, a biochemical process widely employed in beverage manufacturing (Martin & Lindner, 2021), has been gaining traction in the Brazilian market, with fermented beverages like beers and wines asserting their presence, and mead is no exception, becoming increasingly embraced in Brazil (Castro, 2021).

With an alcohol content ranging between 4 and 14 Gay-Lussac degrees (ºGL) at 20°C, mead, as defined by Brazilian legislation, is a beverage resulting from the alcoholic fermentation of a solution composed of bee honey, nutrient salts, and potable water (Brasil, 2009). The standards of identity and quality for mead are stipulated by Normative Instruction No. 34, dated November 29, 2012, which categorizes mead as dry or sweet based on sugar content, expressly prohibiting the use of sucrose in its production (Brasil, 2012a). Moreover, Brazilian legislation does not foresee the use of fruit juices and spices in mead production, a practice observed in other producing countries, but the inclusion of these ingredients should not mask the distinctive flavor and aroma of honey (McConnell & Schramm, 1995).

The Food and Agriculture Organization (FAO) defines probiotics as live microorganisms that, when ingested in appropriate quantities, confer health benefits to the host organism (Mohammad et al., 2020). *Bacillus* strains find a specific niche both in honeybee (*Apis mellifera*) hives and in stingless bee products (Gilliam, 1979). LAB, such as *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Lactococcus*, are widely recognized in the probiotics industry and are considered safe (Generally Recognized As Safe [GRAS] status). Although not widely recognized, *Bacillus* genus bacteria have shown promising results as potential probiotics (Elshaghabee et al., 2017). Furthermore, probiotics play an important role in maintaining intestinal microflora as well as protecting against gastrointestinal pathogens. Over the last 20 years, there has been interesting recognition of their role in intestinal microbiota health, being considered functional bioactive compounds (Brandão et al., 2021).

Studies using pollen to acidify beer have been conducted, for example, in the production of *Catharina Sour*, where a correlation was observed between sensory analysis and liquid chromatography. This showed that the presence of pollen, as well as yeasts, acetic acid bacteria, and fructophilic lactic acid bacteria (FLABs), can contribute to different aromas in these types of beers, as well as enable further studies regarding the characterization of present microorganisms (Ghesti et al., 2023).

This study aimed to create and characterize mead by evaluating the use of pollen from the *Frieseomelitta varia* bee, a stingless bee characteristic of the Cerrado biome. The pollen was utilized as a novel source of *Lactobacillus* in the alcoholic fermentation of mead with the intention of assessing the probiotic potential of its microbiota.

### *1.1 Relevance of the work*

This study presents an innovative contribution to the field of fermented beverages by introducing a novel method of mead production that incorporates the microbiota of *Frieseomelitta varia* bee pollen during fermentation. The use of this specific pollen not only enhances lactic fermentation—modulating the acidity and flavor profile of the final product—but also results in a mead with significant probiotic potential, evidenced by a culture count exceeding $10^6$ colony-forming unit (CFU) $mL^{-1}$. Through standardized physicochemical analyses, improvements were observed in alcohol content, fermentability, color, pH, and organoleptic qualities, aligning with consumer trends favoring functional and lower-alcohol beverages.

By blending traditional mead-making practices with the unique microbiological properties of Brazilian bee pollen, this research offers a distinctive beverage with both sensory appeal and potential health benefits. Additionally, the findings underscore the potential for expanding the Brazilian mead market, leveraging the native biodiversity to create a high-quality, export-ready product. As such, this study not only advances scientific understanding in the field of fermentation but also opens new avenues for innovation in the food and beverage industry.

## 2 MATERIALS AND METHODS

### *2.1 Materials*

For the mead production, 5 kg of honey from The Bees Knees Apiary was used, with a sugar content of 82% measured on the BRIX scale. The composition consisted of 16.5% water with a pH of 4.5. An addition of 26 L of filtered potable water, 20 g of *F. varia* pollen for lactic fermentation, and 10 g of Mangrove Jack's M05 Mead Yeast industrial yeast for alcoholic fermentation was performed. A total of six fermentative reactors were employed. All materials were previously sanitized with iodine and alcohol.

### *2.2 Mead production and analysis*

The process comprises four stages: wort preparation, fermentation, maturation, and bottling. Wort production began by boiling 26 L of filtered potable water until reaching a temperature of 97°C, maintained for 30 min. Afterward, the temperature was allowed to drop to 75°C before adding the honey, and once added, it decreased to 30°C. Original gravity (OG) and final gravity (FG) and pH were measured using standardized methods such as EBC 8.2.2 and EBC 8.17, respectively (Ghesti et al., 2023).

Pollen addition was done in different quantities: concentrations of 1 g/2 L, 5 g/2 L, 10 g/2 L, 20 g/2 L, and an industrial yeast "Rica Nata" of 10 g/2 L. Following the division, bottles were placed in a fermentative reactor to minimize light exposure and allow gas release during fermentation. Additionally, yeast for alcoholic fermentation was added to the remaining 16 L in a 30 L capacity container. The fermentation lasted approximately 24 days. After separating lactic and alcoholic fermentations, both were combined in a 30 L capacity vessel for beverage maturation for about 2 months.

The physicochemical characteristics of mead samples, such as alcohol content and fermentability, were measured using a PBA-B M-AntonPaar equipped with an AlcolyzerPlus

Beer module and a DMA 5000 M density detector (AntonPaar, Austria). The samples were grouped into four different categories: sample with priming and pollen (HPPo), sample without priming and with pollen (HWPo), sample without pollen and with priming (HP), and sample without pollen, without priming (HB). Each sample was analyzed in triplicate, and the average values of each physicochemical parameter were obtained.

The color of the meads was obtained through ultraviolet–visible (UV-vis) spectrophotometry analysis with absorption at 430 nm, following the EBC 9.6 method.

The samples were analyzed using a gas chromatography-mass spectrometry (GC-MS) system, specifically a Shimadzu GC-2010 Plus gas chromatograph coupled with a Shimadzu GCMS-QP2020 MS and a Shimadzu AOC-20i autoinjector (Kyoto, Japan). This system was equipped with an SH-Rxi-5ms capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) and was operated with helium as the carrier gas at a flow rate of 1.06 mL/min. The samples were injected in split mode with a split ratio of 70 and an injection volume of 0.5 µL.

The oven temperature program was as follows: initial temperature at 35°C, held for 5 min; then increased from 35°C to 70°C at a rate of 2°C/min, held for 3 min; followed by an increase from 70°C to 150°C at 10°C/min, held for 1 min; and finally ramped from 150°C to 280°C at 20°C/min, held for 3 min. The MS operated in electron impact mode with an ionization potential of 70 eV and an ion source temperature of 200°C. The interface temperature was set to 300°C. Mass spectrometric scanning was performed in full-scan mode, covering a mass range of 35–500 m/z.

Sensory evaluation was conducted to identify and quantify the organoleptic characteristics of the mead. The analysis took place at the Universidade de Brasília (UnB) with a trained panel of 10 judges. The panelists underwent theoretical and practical training using the FlavorActiv off-flavors kit (Thame, UK), under the supervision of qualified personnel, following the Brazilian Standard NBR ISO 5492:2017 (ABNT, 2017). The sensorial analysis results were categorized into aroma and flavor for each sample. A 5-point grading system was employed, in which 1 represented the lowest and 5 the highest perception intensity. The average scores for each attribute were calculated and visually represented through spider graphs to facilitate comparison across samples.

## 3 RESULTS AND DISCUSSION

Using a hydrometer, the OG was measured as soon as the wort was produced, and after 24 days, the FG was measured, along with pH values for different fermentation stages. The analysis results indicated an OG of 5.2 g/cm$^3$ and an FG of 1 g/cm$^3$, both values corrected to 20°C. Since the final density equals that of water, the fermentation process was successful. For comparison, the FG obtained is similar to that of beer and wine, with values of 1.01 and 1.00 g/cm$^3$, respectively.

Additionally, pH was measured throughout the fermentation process, with readings of 4.2 before fermentation and 3.2 at the end of fermentation, showing that upon finishing the fermentation process, the pH value found is close to that found in wines, between 3.0 and 3.8, depending on the type of wine, cultivation, and vintage (Miele, 2021). In the production of Catharina Sour using pollen, the final pH of the beer was 3.4, illustrating how mead has acidity like other fermented beverages. The color of the meads was obtained using the EBC 9.6 method, and the result obtained was 3.4 for all samples with $p = .05$, which can be characterized as a clear mead, as the value obtained falls within the range of 2–8 EBC.

The results obtained in the analysis of mead and the physicochemical parameters are presented in Table 1.

According to the Table 1, the alcohol content (% v/v of alcohol) fell within the limits established by Normative Instruction No. 34, dated November 29, 2012, which sets minimum and maximum limits of 4 and 14% v/v, respectively.

Based on the calculated apparent degree of fermentation (ADF) and measured density, it can be said that the mead exhibits drier characteristics, with alcohol being more prominent due to the low carbohydrate content in the final sample.

The addition of pollen grains to the mead might improve fermentation rates, alcohol production, and sensory attributes of the beverage, potentially reducing the total acidity in the drink, as there is a slight deviation in yeast metabolism toward the production of organic acids, mainly acetic acid (Roldán et al., 2011).

Furthermore, pollen provides potassium and calcium salts that may contribute to acidity reduction, as observed in Table 1, where acidity records values below those established by Brazilian Legislation, which could, however, be justified by pollen addition and the fact that, due to the absence of a pressurized analysis system, volatile acidity measurement is not performed. In the analyzed samples, it is noticeable that, when compared to the blank (HB), samples with pollen exhibit lower acidity levels (HWPo and HPPo). The use of honey with a higher pollen content results in faster fermentation, as pollen provides nitrogenous compounds for the yeasts (Vidrih & Hribar, 2007). Table 2 shows the results of the GC-MS analyses.

Table 2 shows that the composition of mead is influenced by several factors, including the raw materials used and the fermentation process, which directly impact the formation of volatile compounds. Ethanol was the predominant volatile component in all formulations; however, variations in ethanol content across the different samples suggest that the addition of pollen and priming influences yeast metabolism, altering the efficiency of sugar conversion and the production of secondary metabolites, such as esters and ketones (Brasil et al., 2020).

**Table 1**. Summary of results obtained for the different mead samples.

| Sample | % v/v alcohol | density (g/cm$^3$) | $CO_2$ (mg/L) | ADF (% w/v) | Acidity (mEq/L) |
|---|---|---|---|---|---|
| HB | 8.660 | 0.990 | 15,300 | 99.9 | 35 |
| HP | 8.813 | 0.991 | 19,700 | 99.9 | 40 |
| HWPo | 8.543 | 0.993 | 15,200 | 99.9 | 28 |
| HPPo | 8.653 | 0.993 | 18,967 | 99.9 | 34 |

**Table 2**. Results from GC analyses of the meads.

| | Mead | | | |
|---|---|---|---|---|
| | **HB** | **HWPo** | **HP** | **HPPo** |
| Substance (area%) (similarity%) | Carbon dioxide (0.04) (95) | Isopropyl alcohol (11.00) (96) | Isopropyl alcohol (11.65) (96) | Carbon dioxide (0.09) (95) |
| | Acetaldehyde (0.44) (98) | Acetaldehyde (0.34) (98) | Acetaldehyde (0.56) (98) | Acetaldehyde (0.50) (98) |
| | Ethanol (92.77) (97) | Ethanol (80.54) (98) | Ethanol (81.76) (98) | Ethanol (84.49) (97) |
| | Ethyl acetate (0.29) (98) | Ethyl acetate (2.28) (97) | Ethyl acetate (0.66) (98) | Ethyl acetate (0.55) (97) |
| | Ketoacetic acid (0.02) (80) | 3-methyl-1-butanol (1.92) (98) | 3-methyl-1-butanol (1.76) (98) | 3-methyl-1-butanol (1.22) (97) |
| | 2-methyl-1-propanol (0.54) (89) | 2-methyl-1-propanol (0.68) (95) | 2-methyl-1-propanol (0.67) (96) | 2-methyl-1-propanol (0.33) (97) |
| | - | 2,4,5-trimethyl-1,3-dioxolane (0.04) (84) | 2,4,5-trimethyl-1,3-dioxolane (0.08) (91) | 2,4,5-trimethyl-1,3-dioxolane (0.04) (92) |
| | 2,3-Butanediol (1.24) (97) | 2,3-Butanediol (1.29) (97) | 2,3-Butanediol (0.85) (97) | 2,3-Butanediol (1.23) (97) |
| | - | 2-methyl-1-butanol (0.67) (89) | 2-methyl-1-butanol (0.67) (89) | 2-methyl-1-butanol (0.42) (89) |
| | Glycerin (1.84) (98) | - | Glycerin (0.14) (95) | Glycerin (0.10) (95) |
| | Phenylethyl alcohol (0.57) (96) | Phenylethyl alcohol (0.49) (98) | Phenylethyl alcohol (0.56) (98) | Phenylethyl alcohol (0.39) (98) |
| | - | Coumarin (0.22) (98) | Coumarin (0.12) (96) | Coumarin (0.09) (96) |
| | 1,2,3-Butanetriol (0.08) (87) | - | 1,2,3-Butanetriol (0.05) (84) | Acetic acid (0.09) (96) |
| | 5-acetyldhydro-2(3H)-furanone (0.05) (91) | - | - | 2-hydroxypropanoic acid ethyl ester (0.05) (95) |

The formation of higher alcohols, including 3-methyl-1-butanol, 2-methyl-1-propanol, and 2,3-butanediol, was more pronounced in HWPo and HP. These compounds, known as fusel alcohols, result from amino acid metabolism and contribute to the sensory complexity of the beverage. Similarly, the production of ethyl acetate, a key ester responsible for fruity and solvent-like aromas, was notably higher in HWPo compared to other formulations. The increased formation of this compound in HWPo suggests that the presence of pollen may enhance enzymatic activity related to ester synthesis (Hübbe et al., 2024). Additionally, the exclusive detection of 2-hydroxypropanoic acid ethyl ester in HPPo indicates that the combination of pollen and priming promotes distinct metabolic pathways (Ghesti et al., 2023).

Organic acids, particularly acetic acid, were only detected in HPPo, suggesting that this formulation might have undergone increased oxidative. Likewise, the detection of coumarin exclusively in HWPo, HP, and HPPo suggests that its formation may be linked to the presence of pollen and priming (Ghesti et al., 2023; Hübbe et al., 2024).

The occurrence of cyclic compounds and furanones further highlights the biochemical transformations occurring in different formulations. The identification of 2,4,5-trimethyl-1,3-dioxolane in HWPo, HP, and HPPo suggests that these conditions favor aldol condensation reactions involving alcohols and aldehydes, whereas the exclusive presence of 5-acetyldhydro-2(3H)-furanone in HB indicates a distinct chemical environment (Brasil et al., 2020).

Taken together, these findings demonstrate that the inclusion of pollen and priming alters the metabolic landscape of yeast during mead fermentation, leading to the production of distinct volatile compounds that shape the beverage's sensory attributes. The HB formulation, characterized by a simpler volatile profile, contrasts with HWPo, which exhibited increased ester formation. The HP formulation displayed an intermediate profile, whereas HPPo, combining both pollen and priming, resulted in the most chemically complex composition, including exclusive compounds not detected in the other samples. These results highlight the potential of ingredient modulation in mead production, providing opportunities for tailored sensory profiles and enhanced bioactive properties (Hübbe et al., 2024).

The results of the sensory analysis were divided based on aromas and flavors for each sample, using a rating system from 1 to 5, where 1 represented the lowest and 5 the highest sensory perception, and are summarized in Figure 1.

It was also observed that the samples with priming addition showed a slight increase in alcohol content; however, this was not perceptible sensorially. The presence of pollen reduced the sensation of acidity, as evidenced by the physicochemical acidity values, and presented a more balanced taste characterized by aromas of yellow fruits. Priming did not alter the observed sensory conditions. However, the blank mead (HB) was observed to have a higher alcoholic sensation, as well as higher acidity.

Additionally, samples with added pollen exhibited greater drinkability, with less apparent acidity and notes of yellow fruit aromas, characteristics also observed for *Catharina Sour* beer (Ghesti et al., 2023).

In a study conducted on beer production using *F. varia* pollen, the use of just 15 g of pollen was sufficient to accelerate the acidification process compared to the conventional method, resulting in a beer with a smoother flavor. Furthermore, this study demonstrates how stingless bee pollen can be an efficient alternative in lactic fermentation, speeding up the *kettle sour* process, as well as in the production of *Catharina Sour* with native Cerrado ingredients (Ghesti et al., 2023). Investigations into the composition of the microbiota present in pollen were

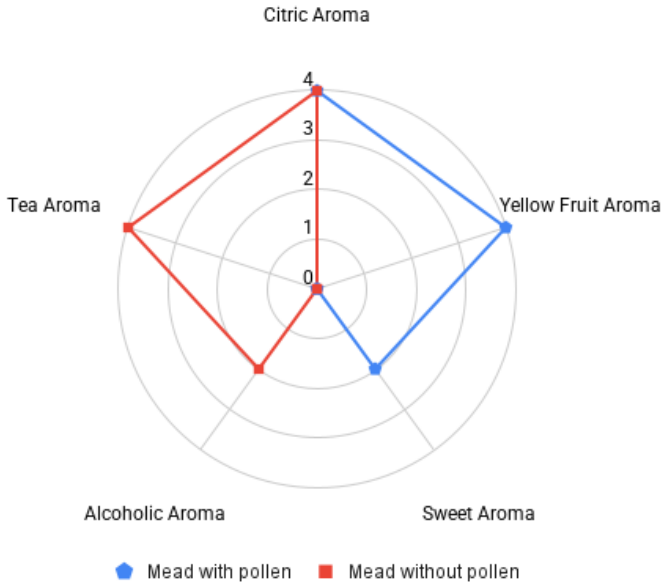


**Figure 1**. Summary of descriptive sensory analysis results.

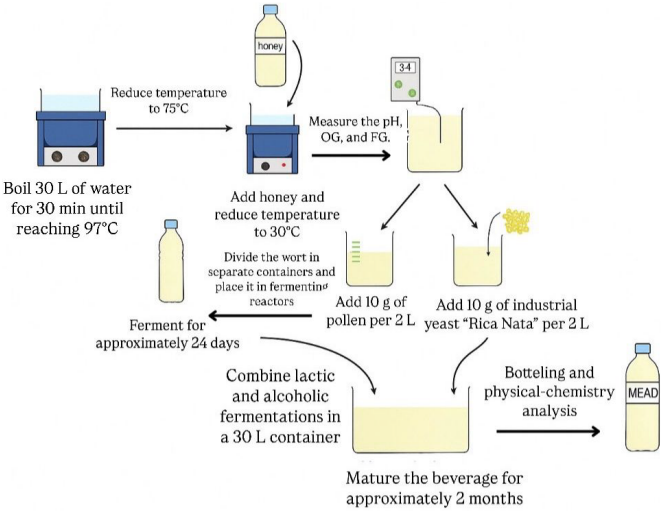


**Figure 2**. Probiotic mead production flowchart.

conducted at the Laboratory of Bioprocess Engineering and Biocatalysts (IB/UnB). The results revealed a significant diversity of microorganisms, with fungi and yeasts being the most prevalent, while only a small proportion consisted of fructophilic bacteria. The analyses pointed to the presence of FLABs, such as *Fructobacillus* and *Lactobacillus*, and yeasts of the genus *Zygosaccharomyces*, from the same family as the yeasts used in alcoholic fermentation (*Saccharomyces*), suggesting that *F. varia* pollen is conducive to microbial activity (Ghesti et al., 2023).

Furthermore, the comparison between the work conducted on mead production and the work on *Catharina Sour* beer production with *F. varia* pollen indicates that the same groups of microorganisms were found, especially FLABs such as *Fructobacillus* and *Lactobacillus*, which may occur knowing that the bees and pollen used are of the same species (Ghesti et al., 2023).

For a beverage to possess probiotic character and exhibit beneficial effects, it is necessary to have a probiotic culture count above $10^6$ colony-forming unit (CFU) $mL^{-1}$ or 6 log CFU $mL^{-1}$ (Brandão et al., 2021). Since the obtained count was above $10^6$ CFU $mL^{-1}$, it is possible to characterize the mead made from pollen as an innovative probiotic beverage.

Based on the experimental results, it can be concluded that fermentation in mead production was successful, bringing forth a new and innovative proposal for a new manufacturing process and suggesting that *F. varia* pollen is an effective substrate for microbial activity and fermentation. This contributes to understanding the potential of stingless bee pollen in the production of fermented beverages and emphasizes the importance of future investigations for a more in-depth analysis of the microbiota present in this material.

Hence, the probiotic mead production could occur according to Figure 2.

## 4 CONCLUSIONS

In this study, an innovative approach to mead production was adopted, exploring microorganisms present in the pollen of the *F. varia* bee during wort fermentation. The addition of pollen played a crucial role in lactic fermentation, adjusting both the flavor and acidity of the mead. This technique allowed us to explore how the pollen microbiota influences the characteristics of the final product, preserving beneficial probiotic strains for health. Using standardized methods for physicochemical analysis, samples were evaluated for alcohol content, fermentability, color, pH, and OG and FG. The results demonstrated that *F. varia* pollen is an effective substrate for microbial activity, producing mead with a probiotic culture count above $10^6$ CFU $mL^{-1}$. Pollen addition improved the physicochemical and organoleptic characteristics of the mead, aligning with market trends for beverages with lower alcohol content and health benefits, offering a new and innovative consumption experience. Although the Brazilian mead market is still in its early stages, its growth potential is significant. This is largely due to the differentiated quality of the product, originating from the combination of Brazilian pollen and honey. Such uniqueness not only expands opportunities for domestic consumption but also positions mead as a product with strong export potential.

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