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Original Research Article

Microbiological Characterization of Honey Based Craft Beer Produced in Northern Côte D'ivoire

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Abstract: Honey-based craft beers were obtained in the towns of Katiola, Korhogo, and Ferkessédougou in northern Côte d'Ivoire. This study aims to evaluate the microbiological quality of a craft beer produced by spontaneous fermentation of honey. To this end, research and counts of microorganisms in raw honey and artisanal honey-based beers were carried out in the different study areas. Microbiological analyses revealed that raw honey contains microorganisms. The results reveal a predominance of fermentative yeasts (6.5±1.14 to 8.6±0.01 log CFU/mL) and lactic acid bacteria (4.5±0.5 to 5.1±0.17 log CFU/mL), confirming mixed fermentation and a total absence of anaerobic sulfite-reducing bacteria. Microorganism analysis showed that during beer production, heating the honey-water mixtures to 100°C for 15 minutes completely eliminated coliforms and enterococci in samples from all locations, and when the mixture was left to ferment for 24 hours without the addition of industrial yeast, natural yeasts developed rapidly (up to 6.9± 0.46 log CFU/mL in Nangakaha). These microorganisms are essential for the production of organic acids, giving the drink microbiological stability and acidity, indicating good production hygiene. This traditional beer could be of interest as a local probiotic product.

Keywords: Craft Beer, Honey, Yeasts, Lactic Acid Bacteria, Fermentation, Ivory Coast.

I-INTRODUCTION

Fermentation is one of the oldest food preservation and processing techniques, particularly in West Africa, where artisanal fermented beverages are of great cultural and nutritional value (Parkouda and *al*., 2007). Honey, rich in fermentable carbohydrates, is an ideal substrate for yeasts and lactic acid bacteria (Schnürer & Magnusson, 2005). The microorganisms involved in these fermentations produce ethanol, organic acids and aromatic compounds, while influencing the microbiological stability of the finished product (Tamang and *al.,* 2016). Few studies have characterized the microbiological profiles of honey based beer in Côte d'Ivoire (Coulibaly and *al*., 2019). Yet this craft beer can be colonized by microorganisms with undesirable characteristics. It is this contact that motivated this study, the aim of which is to characterize the microbial populations of a honey-based beverage produced locally in rural areas in the north of the country. The aspects addressed in this work are the enumeration of aerobic mesophilic germs (AMG), yeasts, lactic acid bacteria, total coliforms, enterococci, and sulfite-reducing anaerobic germs (SRAG).

II- MATERIALS AND METHODS

II-1 Materials

II-1-1 Biological Material

The biological material used consisted of raw honeys and artisanal honey-based beers obtained from producers in Touro (Katiola), Nangakaha (Korhogo) and Lassologo (Ferkessédougou).

II-1-2 Growing Sites

Selective culture media were used to identify aerobic mesophilic germs (AMG), yeasts, lactic acid bacteria, total coliforms, enterococci and sulfite-reducing anaerobic germs (SRAG):

- Plate Count Agar (PCA) medium for the culture of mesophilic aerobic germs (AMG);
- Sabouraud medium for yeast culture;
- MRS (De Man Rogosa and Sharpe) medium for lactic bacteria culture;

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- VRBL medium (Violet Red Bile Lactose Agar) for coliform cultures;
- BEA (Bile Esculin Azide) agar for cultivating Enterococci.
- TSN (Tryptone Sulfite with Neomycin) medium for the culture of sulfite-reducing anaerobes.

II-2 METHODS

II-2-1 Sampling

Samples were taken at various points (raw honey (MB) = critical point 1; honey beer before fermentation (BMavf)= critical point 2; honey beer after fermentation (BMapf) = critical point 3) likely to bring microorganisms contaminating the beers from the various honeys collected in the villages selected at the three (3) sites, namely Touro (Katiola); Nangakaha (Korhogo) and Lassologo (Ferkessédougou). The villages were selected following a survey that took place from July 18 to October 25, 2022, i.e. a week and a half in each village. This survey made it possible to assess the level of knowledge of artisanal honey beer, to list all the producers of this beer among the population, and to record the production diagrams of artisanal honey beer. This study was carried out three times to ensure the reliability of the results. Samples were taken from three producers, one from each locality. Samples were taken aseptically to avoid external contamination. Samples were placed in 500 mL sterile bowls and stored in a cooler containing ice. They were then transported to the Peleforo GON COULIBALY University laboratory for analysis within 6 hours.

II-2-2 Preparation of Stock Suspension and Dilutions

To prepare the honey mother suspension, 10g of the sample was added to 90 mL of sterile peptone water (10^{-1}). For analysis of the beers, these were used directly as the mother suspensions from which decimal dilutions ranging from 10^{-3} to 10^{-8} were made.

II-2-3 Microorganism Detection and Enumeration

- **Aerobic mesophilic germs (AMG)**: Inoculation was carried out on Plate Count Agar (PCA), incubation of inoculated Petri dishes was carried out at 30°C and read every 24 hours for up to 72 hours. Plates containing between 30 and 300 colonies were selected.
- **Yeasts**: Inoculation was carried out on Sabouraud Chloramphenicol agar, and Petri dishes were placed in an oven at 30°C for 24 to 72 hours for incubation. Plates containing between 30 and 300 colonies were selected.
- **Lactic acid bacteria**: Inoculation was carried out on MRS agar (De Man Rogosa and Sharpe). Petri dishes were incubated anaerobically at 30°C for 48 to 72 hours. Plates containing between 30 and 300 colonies were selected.
- **Total coliforms:** Inoculation was carried out by incorporation onto VRBG (Violet Red Bile Lactose Agar), incubation at 30°C - 44°C for 24 hours. Plates containing between 15 and 300 colonies were selected.
- **Enterococci**: Inoculation was carried out on BEA (Bile Esculin Azide) agar, and Petri dishes were placed in an oven at 30°C for 24 to 48 hours for incubation. Characteristic colonies appear as blackish dots.
- **Sulfite-reducing anaerobic germs (SRAG):** Inoculated on TSN (Tryptone Sulfite with Neomycin) agar, tubes are then incubated in an oven at 30°C for 24 to 48 hours, where large black colonies are counted.

II-2-4 Expression of Results

Results are expressed as CFU/ml or CFU/g (Colony Forming Unit per milliliter) or (Colony Forming Unit per gram) and given by the following relationship **[5]:**

$$N = \frac{\sum C}{V_{ml} \times (n_1 + 0{,}1n_2) \times d}$$

V: Volume of inoculum or solution deposited (ml)
n_1: Number of Petri dishes or tubes inoculated at 1st dilution
n_2: Number of plates or tubes inoculated at 2nd dilution
d: Dilution retained or factor of first dilution retained
N: Number of colonies or CFU per g of initial product
CFU/mL: Colony Forming Unit per milliliter
∑C: Sum of colonies of interpretable plates

II-3 Statistical Analysis

One-way variable analysis (ANOVA) and Duncan's test were performed with STATISTICA 99th edition software to understand the variables measured on the different beers. Differences were considered significant for values of ($P < 5\%$). The software was also used to calculate the means and standard deviations of the parameters analyzed. Calculations and figures were made using EXCEL 2016.

III- RESULTS

III-1 Microbial Loads in Raw Honey

Figure 1 shows that all the bacteria tested for were found in raw honey from each location (Touro, Nangakaha, and Lassologo) except for anaerobic sulfite-reducing bacteria. Total coliforms were 1.5 ± 0.3 log CFU/mL; 1.1 ± 0.26 log CFU/mL and 1.3 ± 0.01 log CFU/mL in honey from Touro, Nangakaha and Lassologo, respectively. Regarding enterococci, the load in Nangakaha was 1.2 ± 0.01 log CFU/mL; Touro and Lassologo were 1.4 ± 0.3 log CFU/mL each. Aerobic mesophilic germs (AMG) were the most common in raw honey, with a load of 8.3 ± 0.3 log CFU/mL in Touro, 10.2 ± 0.35 log CFU/mL in Nangakaha, and 8.7 ± 0.56 log CFU/mL in Lassologo. The yeasts found in raw honey also had a high load after the AMG. The yeasts in

raw honey from Touro had a load of 6.5 ± 1.14 log CFU/mL, those from Nangakaha had a load of 8.6 ± 0.01 log CFU/mL, and those from Lassologo had a load of 7.3 ± 0.62 log CFU/mL. The lactic acid bacteria load in raw honey from the different locations was 4.5 ± 0.5 log CFU/mL, 5.1 ± 0.17 CFU/mL, and 4.9 ± 0.6 log CFU/mL in Touro, Nangakaha, and Lassologo, respectively.

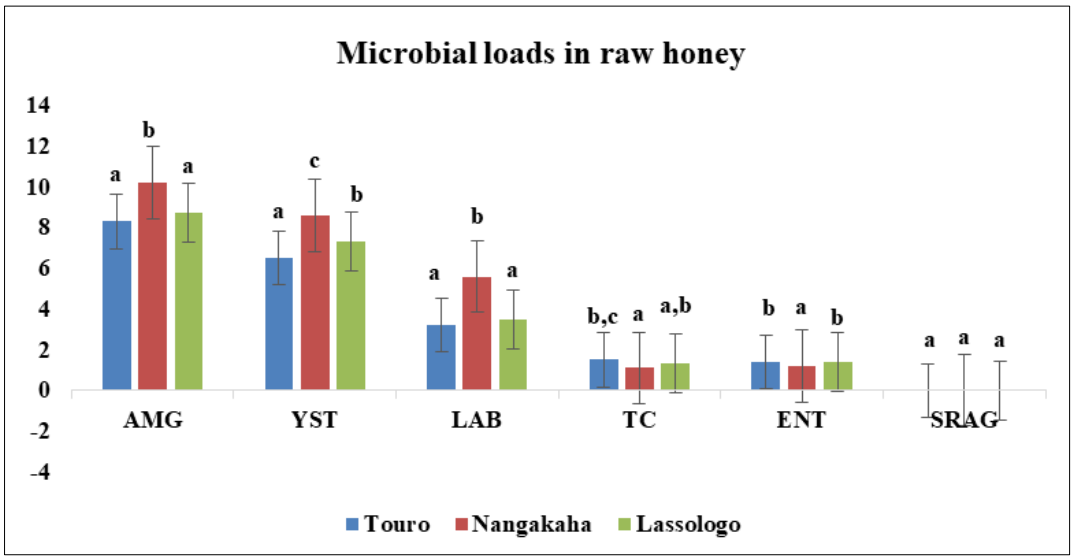

Figure 1: ***Evolution of the microbial load of raw honey***
AMG: aerobic mesophilic germs; **YST:** yeasts; **LAB**: lactic acid bacteria; **TC**: total coliforms; **ENT**: enterococci; **SRAG**: sulfite-reducing anaerobic germs.
Letters **a**, **b** and **c** represent significant differences (P<0.05)

III-2 Microbial Loads in Craft Beers

Figure 2 shows the microorganisms counted in honey-based craft beers sampled at various critical points (before and after fermentation) at producers in each locality (Touro, Nangakaha, and Lassologo).

Before fermentation, artisanal honey beers had the highest AMG loads (8.7 ± 0.2 log CFU/mL) in Nangakaha beer compared to those in Lassologo (7 ± 0.7 log CFU/mL) and Touro (6.3 ± 0.6 log CFU/mL). After 24 hours of fermentation of the same beers, the AMG loads observed in the collected beers were 9.3 ± 0.2 log CFU/mL for the beer from Nangakaha, 7.5 ± 0.5 log CFU/mL for the beer from Lassologo, and 7 ± 0.3 log CFU/mL for the beer from Touro (***Figure 2a***). The yeast loads of the beers produced before fermentation were 4.3 ± 0.52 log CFU/mL in the beer from Touro, 6 ± 0.7 log CFU/mL for the beer from Nangakaha, and 5.2 ± 0.35 log CFU/mL for the beer from Lassologo. After fermentation, they increased to 4.8 ± 0.01 log CFU/mL, 6.9 ± 0.46 log CFU/mL, and 5.4 ± 0.2 log CFU/mL for Touro, Nangakaha, and Lassologo beer, respectively (***Figure 2b***). Before fermentation, the lactic acid bacteria loads observed were 3.2 ± 0.45 log CFU/mL; 4 ± 0.17 log CFU/mL and 3.7 ± 0.2 log CFU/mL for the beers from Touro, Nangakaha and Lassologo, respectively. After fermentation, the lactic acid bacteria counts in the harvested beers increased. Thus, the counts in Touro were 3.4 ± 0.2 log CFU/mL; in Nangakaha 4.6 ± 0.6 log CFU/mL and 4 ± 0.66 log CFU/mL in Lassologo (***Figure 2c***). After boiling the honey-water mixtures at 100°C for 15 minutes, the total coliform and enterococcus counts before and after fermentation were zero in all the artisanal honey beers from each locality.

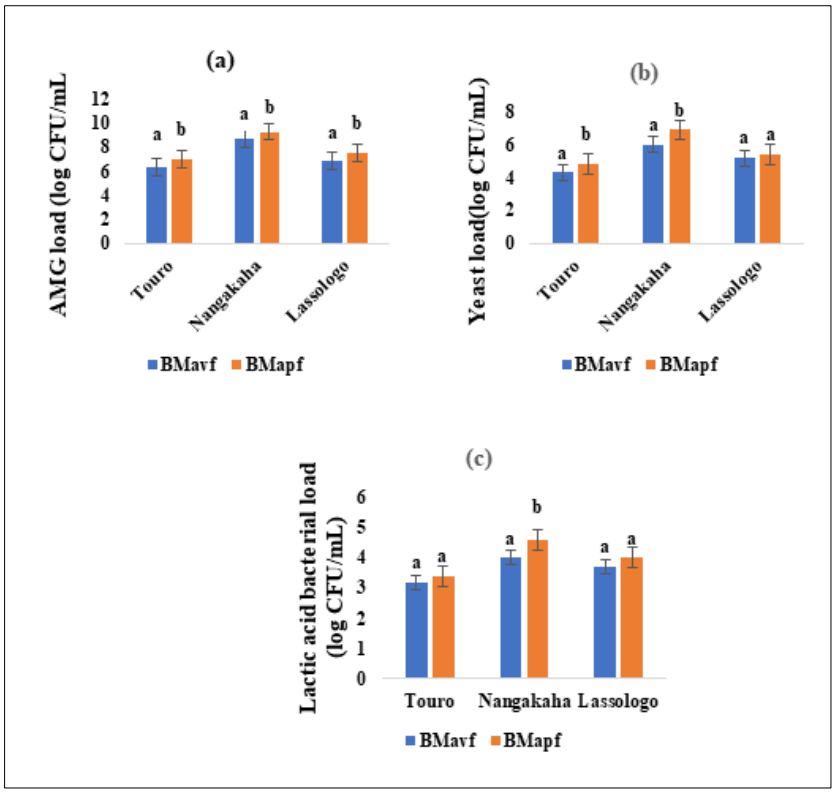

Figure 2: ***Evolution of AMG (a), yeast (b) and lactic bacteria (c) loads in beers from different villages.***
Letters **a**, **b** and **c** represent significant differences (P<0,05)

IV-DISCUSSION

The honey harvested in Touro, Nangakaha, and Lassologo had high levels of mesophilic aerobic bacteria: 8.3 ± 0.3 log CFU/mL in Touro, 10.2 ± 0.35 log CFU/mL in Nangakaha, and 8.7 ± 0.56 log CFU/mL in Lassologo; as well as a significant presence of yeasts (6.5 to 8.6 log CFU/mL). These results indicate significant microbial contamination, probably due to the conditions of extraction, storage, or handling of the honey. Similar results were reported by Parry-Hanson Konadu and *al*., (2020), who observed microbial loads greater than 6 log CFU/mL in artisanal honeys sold in Accra, Ghana, highlighting the vulnerability of raw honey to environmental or human contamination and showing that harvesting hygiene needs to be improved. Total coliforms and enterococci remain modest (≈ 1–1.5 log CFU/mL) and no anaerobic sulfite-reducing bacteria were detected. In contrast, a study conducted in Nigeria by Aminuwa and *al.,* (2024) on honeys from Sudanian zones revealed the absence of coliforms, with a microbial flora dominated by Bacillus spores, suggesting that microbial composition can vary greatly depending on local beekeeping practices. Artisanal honey beers had high GAM loads, particularly in Nangakaha beer (8.7±0.2 log CFU/mL before fermentation and 9.3 ± 0.2 log CFU/mL after fermentation). This post-fermentation increase suggests microbial proliferation during the process, probably due to suboptimal sanitary conditions or cross-contamination. Similar studies on artisanal fermented beverages have also reported high microbial loads, attributed to poorly controlled manufacturing practices (Oscar and *al.*, 2022).

Fermentation, although it generally reduces the microbial load through acidification and alcohol production, can sometimes favor certain resistant microbial populations (Mallari & Simone, 2025). These results highlight the importance of reinforcing good hygiene practices during production. Yeasts play a key role in alcoholic fermentation. In this study, yeast loads were highest in Nangakaha beer (6 ± 0.7 log CFU/mL before fermentation and 6.9 ± 0.46 log CFU/mL after), followed by Lassologo and Touro (Nout & Sarkar, 1999). This variation may reflect differences in indigenous yeast strains or fermentation conditions. Recent work has shown that yeast diversity in artisanal fermentations significantly influences the sensory characteristics and microbiological quality of products (Yu and *al.*, 2022). A moderate increase in yeast after fermentation is expected, but excessively high levels could indicate uncontrolled fermentation, requiring stricter control of parameters (temperature, pH). Bacteria are naturally present in fermentation and can contribute to the flavor and stability of beers. In this study, lactic acid bacteria were more abundant in Nangakaha beer (4 ± 0.17 log CFU/mL before fermentation and 4.6 ± 0.6 log CFU/mL after). This increase is consistent with their role in fermentation ecosystems, where they proliferate in synergy with yeasts (Anumudu, 2023). However, high levels of lactic acid bacteria can also lead to excessive acidification, impairing product quality. Studies

recommend monitoring these populations to avoid microbial imbalances (Holzapfel and *al*., 1998). Heating honey-water mixtures to 100°C for 15 minutes completely eliminated coliforms and enterococci in samples from all locations, which is consistent with the work of Snowdon and *al*. (2004), according to which high-temperature heat treatment eliminates pathogenic flora without significantly altering the honey.

V- CONCLUSION

The results highlight significant microbiological variations between beers from different villages, reflecting distinct local practices and microbial ecosystems. To improve the quality and safety of craft beers, it is essential to adopt standardized hygiene and fermentation protocols, while preserving traditional characteristics. These traditional beverages could be valorized as functional foods of local origin. Further research could explore the impact of these microorganisms on the sensory and nutritional properties of beers.

Acknowledgments

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