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Identification of molds and the influence of physicochemical factors in attiéké from Burkina Faso

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This cross-sectional study identified mold contamination and its relationship with physicochemical factors in attiéké sold in Burkina Faso. Over two months (February-March 2023), coinciding with the cool-to-hot dry season transition, 100 attiéké samples were collected from markets in three major cities (Ouagadougou, Bobo-Dioulasso, and Koudougou). Fungal cultures were established on Sabouraud medium supplemented with chloramphenicol. Positive cultures were then counted, and the isolated molds were identified phenotypically. Moisture content and pH were measured using standard methods to assess their influence on fungal contamination. ANOVA ($p < 0.05$) identified significant differences in variables between cities, while Pearson correlation analysis was conducted to explore the relationships between physicochemical factors and fungal contamination levels. All samples (100%) exhibited fungal contamination at varying levels. The results revealed that *Aspergillus* (47.62%) was the most common mold, followed by *Penicillium* (32.14%), *Fusarium* (17.86%), and *Mucor* (2.38%). The average yeast and mold load was 1.4 x 10^5 CFU/g, with the highest contamination observed in Bobo-Dioulasso. The mean pH and moisture content were 5.14 ± 1.10 and 53.26 ± 4.15, respectively. The results revealed a significant correlation between physicochemical factors and microbial load ($p < 0.05$). Thorough testing during production is crucial for detecting mycotoxin contamination, evaluating its potential harm, and ensuring product safety.

Key words: *Aspergillus*, moisture content, mycotoxin, contamination, mold, pH.

INTRODUCTION

Attiéké, a fermented cassava semolina, is a widely consumed and valued traditional dish in Burkina Faso (Guira et al., 2016). However, the presence of mold can compromise its quality and safety, posing potential public

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Table 1. Sampling procedures and data collected.

Sample type	Ouagadougou	Bobo-Dioulasso	Koudougou
Attiéké sold at retail	50	25	25

health risks. Its production involves several post-harvest processing steps, such as peeling, steaming, fermentation, and spinning (Gnagne et al., 2016). Although food safety regulations are becoming increasingly stringent in many countries to protect consumers from chemical and mycotoxin contamination (Logrieco et al., 2018), controlling processing conditions, particularly in hot and humid tropical environments, remains challenging (Montet, 2022). This challenge is further amplified by the unpredictable nature of mold contamination, influenced by a complex interplay of factors like temperature, humidity, storage time, and the presence of native fungal flora (Kouadio et al., 2015). Mold contamination is particularly concerning because some molds, like *Aspergillus* and *Penicillium*, can produce harmful mycotoxins such as ochratoxin A (Khaldi et al., 2020; Compaoré et al., 2021). Despite efforts by producers, finished products often show the presence of yeasts and molds, potentially exceeding safety limits due to inadequate packaging and storage conditions (Yobouet, 2016; Onodugo et al., 2023). Given the potential health risks of mycotoxins and the limited data on mold contamination in Burkina Faso's attiéké production chain, this study aimed to isolate and identify the mold genera present in attiéké and analyze its physicochemical parameters to assess its safety and marketability.

MATERIALS AND METHODS

Study location

This cross-sectional study was conducted from February to March 2023 in three major cities in Burkina Faso: Ouagadougou (12°21'58" N, 1°31'05" W), Bobo-Dioulasso (11°11'00" N, 4°17'00" W), and Koudougou (12°15'04" N, 2°22'28" W). These cities are located in the Central (Kadiogo Province), Hauts-Bassins (Houet Province), and Central West (Boulkiemdé Province) regions, respectively. During this period, the minimum temperatures were 18°C in Bobo-Dioulasso, 19°C in Ouagadougou, and 27°C in Koudougou.

Sample collection

A total of 100 attiéké samples were collected from these cities, representing individually sold portions at public markets (Table 1). To ensure a representative sample, bulk purchases of 500 g of attiéké were divided into smaller portions, mimicking individual sales. Physicochemical analyses were carried out at the Laboratory of Applied Biochemistry and Immunology (LaBIA), while microbiological analyses were performed at the Laboratory of Molecular Biology, Epidemiology, and Surveillance of Bacteria and Viruses Transmissible by Water and Food (LaBESTA), both located at Université Joseph KI-ZERBO.

Microbiological analysis

Isolation

The detection of yeasts and molds in the attiéké samples was carried out according to the standard ISO 21527-2 (2008). A volume of 100 µl of the mother suspension, as well as successive dilutions, was inoculated onto Sabouraud chloramphenicol agar (Liofilchem, Italy), and the plates were then incubated at 25°C for 5 days. Yeasts produced creamy white colonies, while molds formed filamentous colonies of various colors on Sabouraud chloramphenicol agar.

Enumeration of yeasts and molds

Colonies were counted after each microorganism had completed its incubation period. Results were expressed in colony-forming units per gram (CFU/g). The number N of microorganisms present in each analyzed sample was evaluated according to the criteria described in ISO 7218 (2007). Colonies were considered countable if their numbers ranged between 4 and 300 for two successive dilutions. To determine the exact number of colonies, the following formula was used:

$$N = \frac{\Sigma C}{V(n1 + 0.1n2)d}$$

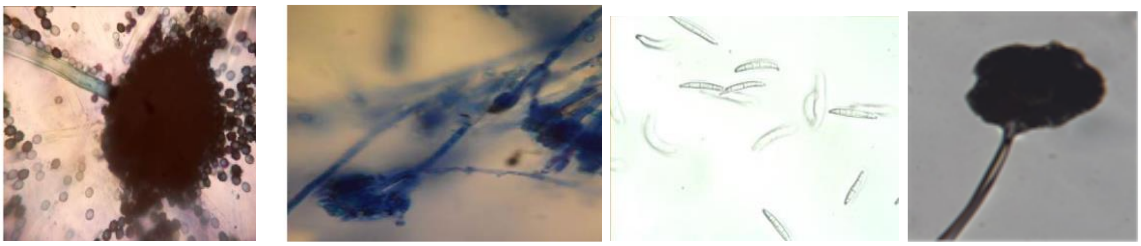
where ΣC: sum of colonies counted on the two boxes retained from 2 successive dilutions, V: volume of inoculum inoculated, d: dilution from which the first counts are retained, n_1: number of boxes at the 1st dilution, n_2: number of boxes at the 2nd dilution.

Interpretation of the microbiological results

A three-tier classification system was used for the microbial analysis. Samples with results below or up to three times the threshold value were considered microbiologically satisfactory. Samples with results between three and ten times the threshold were considered acceptable. Results exceeding ten times the threshold were classified as having unsatisfactory microbiological quality. The criteria used were: a criterion m=5.10^2 CFU/g and a maximum threshold (M) =5.10^3 CFU/g.

Identification of molds and yeasts

Macroscopic: The appearance of each colony was observed using naked-eye identification criteria, including the color of the isolate and its reverse side, as well as appearance and pigmentation (Compaoré, 2022). On Sabouraud chloramphenicol medium, molds appeared in various colors ranging from white to cream, pink, green, and even black.



Aspergillus spp. *Penicillium* spp. *Fusarium* spp. *Mucor* spp.

Figure 1. Microscopic appearance of fungal strains (×100).

Their size varied from very small to large, with colony shapes ranging from round to irregular, sometimes with regular, jagged, or irregular edges. Additionally, they exhibited various textures such as velvety, cottony, smooth, wrinkled, bumpy, or grainy, depending on the genus, temperature, humidity, nutrient availability, and oxygen levels. In contrast, yeasts appeared in shades of cream, white, or beige, with small colonies that had a smooth, uniform surface, differentiating them from mold colonies, which were often more fluffy or rough.

Microscopic: Microscopic observation of fungal colonies was carried out using the slide culture method. A mycelial fragment was placed on a clean slide with a drop of distilled water. The fragment was then gently teased apart using a platinum needle to avoid excessive density. Finally, a coverslip was carefully placed to avoid the formation of air bubbles or overflows. This technique allows for the observation of the thallus, its septate or non-septate nature, as well as the characteristics of conidiospores and spores (Dufresne and Guy, 2018). Microscopic observation was carried out at x40 and x100 magnifications using a standard binocular microscope (Leica, Germany). Identification was performed according to the methods described by Dufresne and Guy (2018) and Waré (2018).

Determination of physicochemical parameters

Determination of moisture content

Moisture content in the attiéké samples was determined according to the method described by Fontana and Carter (2020). A 5 g test portion of attiéké was placed in a calibrated boat and weighed before the test. The sample was then introduced into an oven (Memmert, Germany) set at 105°C ± 2°C until a constant mass was achieved. The sample was placed in the oven for 3 h, then cooled in a desiccator for 15 min. After cooling, the boats were weighed using a high-precision electronic balance (Yoke Instruments, China). This process was repeated several times, placing the boats back in the oven for 30-min intervals until the mass remained stable. The moisture content (TH%) was calculated by the relative difference in the mass of the test sample before and after drying in the oven (Nielsen, 2010).

$$\boldsymbol{TH}\ (\%) = \frac{\mathbf{PE} - (\mathbf{Pf} - \mathbf{P0})}{\mathbf{PE}} \times \mathbf{100}$$

where PE: Test portion (5 g), P0: weight of the empty crucible in grams, Pf: weight of the crucible containing the dry sample (final weight) after passing through the oven.

pH measurement

The pH of the attiéké samples was measured according to the method described by AOAC (2012). This measurement was conducted by immersing the glass electrode of a pH meter (Hanna Instruments, USA), which had been previously calibrated with buffer solutions of pH 7 and 4 (Amoa-Awua et al., 2007), into 10 mL of supernatant. The pH value displayed on the pH meter screen was recorded. Two measurements were performed for each sample to obtain an average value.

Statistical analysis

The data processing was conducted using SPSS version 20 and Excel 2019 software. Quantitative variables were expressed as mean (x) ± standard deviation (s), with extreme values (minimum and maximum) indicated. Qualitative variables were presented in numbers and percentages. The χ^2 test was used for comparing variables with (k-1) degrees of freedom, with a significance threshold of 5%. The ANOVA test was used to compare the means. Additionally, Pearson correlation analysis (R function: “co”) was performed to examine the relationships between moisture content, fungal contamination, and pH of attiéké.

RESULTS

Microbiological parameters

Study of fungal microflora

Microscopic and macroscopic characteristics were examined to identify fungal strains isolated from 100 attiéké samples sold at retail outlets. The analysis focused on color, colony appearance, the reverse side of the colonies, thallus shape, and spores. This initial screening revealed four predominant fungal genera: *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*.

Characteristics of isolated fungal strains

Morphological observations of 84 fungal strains revealed the prevalence of *Aspergillus* (47.62%), *Penicillium* (32.14%), *Fusarium* (17.86%), and *Mucor* (2.38%) genera. Figure 1 displays representative mold strains observed under a binocular microscope (Leica, Germany)

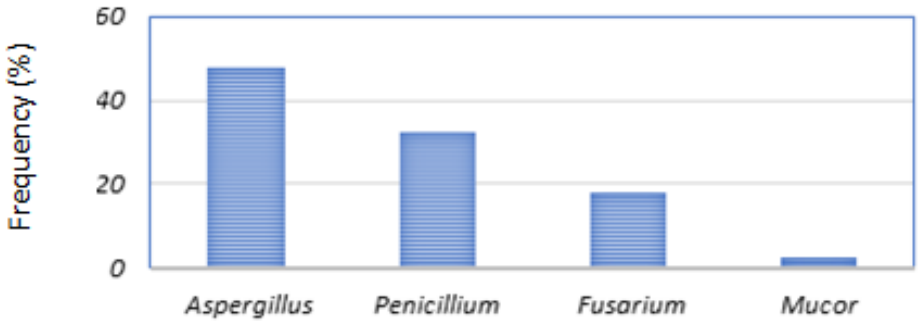


Figure 2. Microflora of attiéké samples detected on medium.

Table 2. Microbiological qualities of samples.

Sample/Locality	Load (CFU/g)
Ouagadougou (n=50)	$0.5.10^5 \pm 0.79^a$
Bobo-Dioulasso (n=25)	$1.4.10^5 \pm 0.58^b$
Koudougou (n=25)	$1.1.10^5 \pm 0.82^b$
Average	$1.1.10^5 \pm 0.77$
Extreme values	5.10^2 - $3.2.10^5$

Different superscript letters within the column indicate significant differences at $p \leq 0.05$.

Table 3. Appreciation values of *attiéké* (n= 100).

Three-class criteria	Yeasts and molds
Satisfying	22 (22)
Acceptable	11 (11)
Not satisfaisant	67 (67)

at ×40 and ×100 magnifications. The proportions of each genus are depicted in Figure 2. Genus identification was facilitated using figurative keys, dichotomous keys, and specific mold characteristics.

However, it is important to note that morphological characteristics alone may not be sufficient for definitive species identification within these genera. Further analysis using molecular techniques could provide more precise species-level identification.

Evaluation of the quality of attiéké

The mean fungal loads in attiéké samples from Bobo-Dioulasso (1.4×10^5 CFU/g) were significantly higher than those from Ouagadougou (0.5×10^5 CFU/g) and Koudougou (1.1×10^5 CFU/g) (Table 2). Statistical analysis revealed no significant difference between Koudougou and Bobo-Dioulasso ($p = 0.7$). However, Ouagadougou exhibited significantly higher contamination levels than both Bobo-Dioulasso and Koudougou ($p = 0.007$ and $p < 0.001$, respectively). More importantly, a high total non-satisfaction rate of 67% was observed across all analyzed attiéké samples (Table 3), suggesting that a substantial proportion of the attiéké may not meet quality standards for mold contamination.

Physicochemical parameters

The physicochemical analysis results of the attiéké samples are summarized in Table 4. The pH values ranged from 3.10 to 9.03, with a mean of 5.14 ± 1.10. It is important to note that this pH range falls within the optimal range for mold proliferation (pH 3-8). The moisture content of the ready-to-eat attiéké ranged from 24.85 to 58.55%, with an average of 53.26 ± 4.15%. This variability in moisture content could influence the growth of mold and other microorganisms. Samples from Koudougou and Bobo-Dioulasso had similar pH values ($p = 0.86$). In contrast, the pH of samples from Ouagadougou differed significantly from those of Bobo-Dioulasso ($p = 0.006$) and Koudougou ($p = 0.011$). No significant variation ($p > 0.05$) was observed in the moisture content of attiéké among the three cities studied.

DISCUSSION

The attiéké production process involves various ferments and unit operations that impact the final product's quality. Contamination during production can be influenced by factors such as initial microflora load, high humidity, pH, and storage temperature (Osaili et al., 2023; Karanth et al., 2023). Physicochemical analysis showed that the pH of the different samples ranged from 3.10 to 9.03, with an average of 5.14 ± 1.10. This pH range provides favorable conditions for mold proliferation, as fungi can grow between pH 3 and 8, with an optimum growth range of 5 to 6 (Ferhi and Ghaia, 2020). Additionally, higher moisture levels in samples correlated with increased contamination, as most molds prefer elevated humidity

Table 4. Physicochemical properties of attiéké samples.

Locality settings	pH	Moisture content (%)
Ouagadougou (n=50)	4.76±1.01[b]	53.10±5.48[b]
Bobo-Dioulasso (n=25)	5.46±0.86[a]	53.43±2.20[a]
Koudougou (n=25)	5.62±1.23[a]	53.40±2.20[a]
Average (n=100)	5.14 ±1.10	53.26±4.15

Values with the same letter in the same column do not differ significantly (p ≤ 5%) according to post-hoc tests.

levels (Chenqiu et al., 2021). Water activity, a critical factor in mold growth and toxin production (Tapia et al., 2020), was relatively low in the samples compared to similar cassava-based foods from Ivory Coast (Yéboué et al., 2017; Adaye, 2020). This lower moisture content contributes to product preservation by limiting microbial growth (Adaye, 2020). Overall, attiéké consumed in the main cities of Burkina Faso exhibited favorable physicochemical characteristics in terms of pH, acidity, dry matter, and moisture content. However, high levels of fungal contamination were observed in certain samples, posing a risk of food poisoning (Abbas et al., 2019). This contamination likely stems from the degradation of raw materials during production due to improper storage conditions. Yeast contamination is common in food products, including attiéké, as yeast is ubiquitous in the environment (Kouadio-Yapo et al., 2018).

Studies have also reported molds and yeast in various street foods (Nkenna, 2023; Barreira et al., 2024), though some regions may show lower levels or even an absence of contamination (El Marnissi et al., 2012). Contaminants can originate from the sales environment, food handlers, or preparation tools (Mahunu et al., 2024; Salamah et al., 2024).

Yeasts are particularly detrimental to attiéké quality (Guira et al., 2021), and molds can produce toxins harmful to consumers (Adeoye et al., 2023). The prevalence of *Aspergillus* in contaminated food, particularly cereals, is well-documented (Oyebamiji et al., 2023; Kortei et al., 2023). *Fusarium* isolates are naturally found in field crops and soil (Compaoré, 2022). Variations in mold levels in attiéké were observed across study areas, consistent with previous findings (Cisse et al., 2023). These variations were likely influenced by climatic conditions, storage practices, and initial fungal load (Chenqiu et al., 2021; Emekwuru and Ejohwomu, 2023; Daba et al., 2024). The higher humidity in the Bobo-Dioulasso area may explain the elevated contamination rates. In conclusion, the high contamination rate in attiéké samples highlights the need for improved production and storage practices to ensure food safety.

Conclusion

Attiéké consumed in the main cities of Burkina Faso is heavily contaminated by molds, notably *Aspergillus*, a toxin-producing genus. This alarming level of contamination poses significant public health risks, as *Aspergillus* can produce mycotoxins linked to various diseases, including liver cancer, respiratory problems, and immune suppression. This raises public health concerns and reveals inadequacies in current quality control. High humidity levels, averaging 53.26 ± 4.15%, facilitated the growth of *Aspergillus* mold, while the observed pH levels, averaging 5.14 ± 1.10, were insufficient to inhibit fungal proliferation. Although the scope of this study is limited, it highlights the urgent need for stricter quality control measures across all stages of the production chain, including close monitoring of key factors such as pH and hygiene practices. Implementing these improvements is essential for protecting consumer health and ensuring that attiéké remains safe for all consumers in Burkina Faso.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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