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Physicochemical and microbiological characterization of flours from the local variety of purple corn (*Zea mays* L.) produced and marketed in Katiola (Côte d'Ivoire)

Moumouny Traore*[1], N´Zebo Desiré Kouame[2], Pepiesin Marie Ange Melem Soro[3], Zamblé Bi Irié Abel Boli[3]

[1]*Department of Biological Sciences, Faculty of Biochemistry and Genetics, Laboratory of Food Microbiology and Biotechnology, Peleforo Gon Coulibaly University, Kohogo, Côte d'Ivoire*
[2]*Departement of Biosciences, Faculty of Biotechnology Agriculture and Valorization of Biological Resource, Felix Houphouet Boigny University, Abidjan, Côte d'Ivoire*
[3]*Department of Food Science and Technology, Faculity of Food Biotechnology and Microbiology, Nangui Abrogoua University, Abidjan, Côte d'Ivoire*

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ABSTRACT

Purple corn is used to prepare many popular dishes. This study involved physicochemical and microbiological analyses of purple flour samples. The aim was to improve the sanitary quality of this popular flour. A total of 71 samples, each weighing approximately 250 g, were collected from fifteen randomly selected vendors at the main market in Katiola (8° 8' 26.002" N, 5° 6' 7.999" W). The samples were labeled, placed in sterile screw-top jars, sealed, and transported to the laboratory. All analyses were performed according to established standards. The pH of the samples ranged from 4.16 to 4.26, indicating an acidic environment. Titratable acidity was low, ranging from 0.14 to 0.16. The moisture content ranged from 11.66 to 11.75%. All of these measured parameters were within the limits set by the standards. The Mesophilic aerobic bacteria load ranged from 2.7 to 6.7×10^5 CFU/g. The total coliform and thermotolerant coliform loads were between 4.4 and 5.8×10^3 CFU/g and between 1.5 and 3.6×10^3 CFU/g, respectively. The *Staphylococcus aureus* load ranged from 4.5 to 5.7×10^3 CFU/g. The contamination levels of these bacteria were all above the thresholds set by the standards. The Mold load varied between 2.1 and 5×10^4 and was in line with the microbiological criterion of 10^4 to 5×10^4 CFU/g. The three species of fungi identified were all of the genus Aspergillus. ASR and *E. coli* were not detected in the analyzed samples. Efforts are needed to improve the quality of this popular flour.

***Corresponding author:** Moumouny Traore ✉ traoremoumouny@yahoo.fr
* https://orcid.org/0009-0000-6816-6742

Co-authors:
N'Zebo Desire Kouame: https://orcid.org/0009-0004-2956-4392
Pepiesin M. A. Melem Soro: https://orcid.org/0009-0009-2741-8945
Zamblé Bi Irié Abel Boli: https://orcid.org/0009-0006-6817-3591

INTRODUCTION

Corn (*Zea mays* L.), native to Central America, belongs to the Poaceae family and the Panicoideae subfamily. It was introduced to West Africa in the 16th century (Domínguez, 2024). Corn cultivation plays a key role in the supply of cereals. In fact, corn is the world's leading cereal crop, with an estimated production of over 1.238 billion tons (CIC, 2024).

African corn production is estimated at 98,819,000 tons, accounting for 5% of the global total. Ivorian corn production was approximately 1.2 million tons in 2023. In Côte d'Ivoire, corn (*Zea mays* L.) is the second most cultivated cereal crop after rice (FAOSTAT, 2025).

Maize is rich in starch, making up 72% to 75% of the grain's mass, as well as protein, making up 8% to 11%, and minerals such as potassium, phytate, and magnesium (Dié *et al.*, 2019).

However, corn proteins are low in essential amino acids, such as lysine and tryptophan (Goredema-Matongera *et al.*, 2021; Akoun, 2022). Therefore, consuming only corn can lead to malnutrition. Corn is used for silage and animal feed. In the medical field and across various industrial sectors, corn is used to manufacture bioplastics, bioethanol, modified starches, and other products (Reddy and Yang, 2005). In several Sub-Saharan African countries, *Zea mays* L. is primarily used for direct human consumption, accounting for up to 60% of daily calorie intake (Takpa, 2022).

In Côte d'Ivoire, the National Agricultural Research Center (CNRA) has made several improved corn varieties available to farmers. In addition to these varieties, a traditional variety known as "purple corn" has been preserved in the center of the country, specifically in the Hambol region (Katiola and the surrounding area). In this region, purple corn is the most widely grown variety, at 47%, compared to 43% for yellow corn and 10% for white corn.

Three local variants of purple corn are found in the Katiola area: Dark purple corn (Nandé wô), light purple corn, and light purple corn with a reddish tinge (Nandé n'kon) (N'Da *et al.*, 2014; N'Da *et al.*, 2023).

Purple corn is a natural source of anthocyanins, which help prevent cardiovascular disease and slow cellular aging. They also reduce LDL cholesterol and normalize blood pressure.

Purple corn boasts excellent organoleptic qualities, as well as therapeutic and sociocultural benefits Anthocyanins, natural pigments, are responsible for its color. The high levels of anthocyanins in purple corn flour are beneficial for eye and vascular health. It is gluten-free and is a powerful food coloring agent (Chaiittianan *et al.*, 2017; Akaffou *et al.*, 2018 ; N'Da *et al.*, 2023). Furthermore, purple corn matures early and commands a higher price than other varieties grown in the region. For the local population, purple corn is a staple food. Its flour is an essential ingredient in the preparation of a wide variety of highly regarded dishes. These include "Tôh" or "Cabato" (cooked gelatinized paste), the alcoholic beverage "Tchapalo," the non-alcoholic beverage "Zoom-Koom," couscous ("Bashi"), fritters, pancakes, and "Koko Baca," a porridge for children and adults (Sika *et al.*, 2019; Coulibaly *et al.*, 2025).

Studies have been conducted in Côte d'Ivoire on the genetic and morphological diversity, as well as the biochemical and nutritional composition, of purple corn. However, few studies have examined the microbiological quality of purple corn flour. Despite its good organoleptic qualities and therapeutic and sociocultural virtues, the flour may be a source of contamination due to its artisanal production, and sales and storage conditions. Thus, this study aims to promote the health and safety of purple corn flour sold in Katiola markets.

MATERIALS AND METHODS

Biological material

The biological material used in the study was purple corn (*Zea mays* L.) flour (Fig. 1).

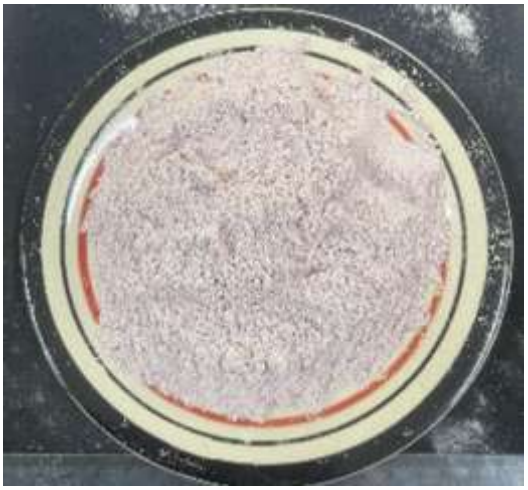

Fig. 1. Photograph of corn flour

Technical equipment

This study used standard microbiology laboratory equipment. This included an electronic scale for weighing materials, an autoclave (Lequeux, France) for sterilizing culture media, ovens (Memmert, Germany) for incubating microorganisms, water baths (Heidolph, Germany) for regenerating solid culture media, and a microscope for viewing fungal isolates. A pH meter was used to adjust the pH, and a refrigerator was used to store materials.

Sampling

Samples were collected in the Hambol region, specifically in the municipality of Katiola (8°8'26.002" N, 5°6'7.999" W). Purple corn is a traditional variety native to this region.

Between April 9 and May 30, 2025, purple flour samples were collected from fifteen vendors at the town's main market. The vendors were randomly selected from the town's main market.

A total of 71 samples, each weighing approximately 250 g, were collected. The samples were labeled, placed in sterile, hermetically sealed, screw-top jars, and transported to the laboratory.

Physicochemical parameters

pH

In an Erlenmeyer flask containing 25 mL of distilled water, 5 g of each flour sample was added. The mixture was then homogenized and filtered. The calibrated pH meter was then immersed in the suspension. After stabilization, the pH was read on the meter's screen.

Titratable acidity

In an Erlenmeyer flask containing 25 mL of distilled water, 5 g of each flour sample was added. The mixture was homogenized and filtered. After adding two to three drops of phenolphthalein to the suspension, the solution was titrated with 0.1 N NaOH until a persistent pink color was obtained. Titratable acidity (%A) was determined using the following formula:

$A\% = V_{NaOH} \times C_{NAOH} \times 0.09 \times 100 / Vech$

C_{NaOH}: Concentration of the NaOH solution ; V_{NaOH}: Volume of NaOH ; 0.09: Milli equivalents of lactic acid per gram; Vech: Volume of the sample.

The moisture content was determined by measuring the empty weight of the capsules with a calibrated balance, followed by measuring the weight of the capsules containing 5 g of the sample. The capsules containing the sample were placed in an autoclave at 103°C for three hours. After removing the capsules from the autoclave, they were weighed again. The moisture content was determined using the following formula.

Humidity H (%) = PE – (PF-PV) / PE × 100

Caption: empty weight of the dried capsule (PV), sample weight (PE), final weight of the capsule + dehydrated sample (PF).

Microbiological analyses

Preparation of stock solution and decimal dilutions (ISO 6887 :2017)

Add 10 g of flour to 90 mL of buffered peptone water (BPW). The homogenized mixture constituted the stock suspension. Tubes containing 9 mL of BPW were prepared. One milliliter of the stock suspension was added to the first tube. After homogenization, the resulting suspension constituted the 10^{-1} dilution. One milliliter of the 10^{-1} dilution was transferred to a

second tube containing 9 milliliters of BPW. This resulted in the 10^{-2} dilution. The same process was repeated under the same conditions to obtain the working dilutions, with a new tip used for each step.

Mesophilic aerobic bacteria (MAB) were counted using the NF ISO 4833:2003 method. One milliliter of the stock, suspension, and dilutions (10^{-2}, and 10^{-3}) was seeded on the surface of the agar that had already been poured into Petri dishes. The seeded dishes were then incubated at 30°C for 72 hours.

Staphylococcus aureus was counted on Baird Parker Medium (BP OXOID CM0275) in accordance with the 1999 standard NF EN ISO 6888-1. Baird Parker agar with added egg yolk emulsion was poured into Petri dishes. After solidification, 0.1 mL of the stock suspension and dilutions (10^{-2} and 10^{-3}) were spread on the surface of the dishes. The dishes were then incubated at 37°C for 24 to 48 hours.

Fecal coliforms were counted using the NF V08-050 method, and total coliforms were counted using the NF ISO 4832 method, both on VRBL agar. One milliliter of the stock suspension and dilutions (10^{-2} and 10^{-3}) were seeded into the mass. The inoculated Petri dishes were incubated for 24 hours at 37°C for fecal coliforms and 44°C for total coliforms.

The *Escherichia coli* count was performed according to the NF ISO 16649-2 method on Rapid *E. coli* medium, which was then incubated at 44 °C for 24 and 48 hours.

Anaerobic sulfite-reducing organisms (ASR) counting was performed in accordance with ISO 15213 :2003. Dilutions (10^{-1} and 10^{-2}) were added to liquid TSN medium that had cooled but not solidified. After solidification, a second layer of uninoculated medium was added to the surface. This step creates anaerobic conditions. The tubes were then incubated at 37°C for 24 hours, after which the black colonies were counted.

Mold and yeast counting and identification

Molds were detected using the NF V08-059 :2002 method on PDA (potato dextrose agar) medium. A 0.1-mL spread of the stock suspension and dilutions (10^{-2} and 10^{-3}) was made on the surface of the poured agar in Petri dishes. The seeded Petri dishes were then incubated at 30°C for two to four days. After incubation, varied mycoflora developed. The strains were then transferred to PDA medium for purification. Macroscopic and microscopic observations enabled presumptive identification based on the proposed identification keys (Bendjoudi and Dehimi, 2020).

Determination of bacterial loads

Counting was performed in accordance with ISO 7218 :2007. The results were expressed as colony-forming units (CFU) per gram using the following formula:

$$N = (\Sigma Colonies)/(V\ mL \times (n_1 + 0{,}1\ n_2) \times D)$$

ΣC: sum of colonies on all plates of the two successive dilutions ; V: volume of inoculum ; n_1 and n_2: number of plates for the first and second dilutions, respectively ; d: dilution rate of the first plate producing countable colonies (low dilution) N is expressed in CFU/mL.

Statistical analyses

The results were analyzed using R software, version 4.3.1, for robust statistical analysis. This enabled the calculation of means and standard deviations for the various microbial loads across samples. Tukey's test was used to compare the means, considering statistically significant variability for probabilities below 0.05.

RESULTS

The pH, titratable acidity, and moisture content of the analyzed flour samples are given in Table 1. The pH of the flours was acidic, ranging from 4.16 to 4.26. Titratable acidity was low and varied minimally (0.14-0.16). The average moisture content ranged from 11.66% to 11.75%. All of the measured parameter values were within the limits set by the standards. No significant differences were observed among the samples for pH, titratable acidity, and moisture content.

Table 1. pH, titratable acidity, and moisture content of the analyzed flours

	Minimum	Maximum	Normal values
pH	4,16 ± 0,15[a]	4,26 ± 0,05[a]	5,5
Titratable acidity (%A)	0,14 ± 0,0001[b]	0,16 ± 0,0000[a]	0,25-0,04%
Moisture content (%H)	11,66 ± 0,0057[a]	11,75 ± 0,0500[a]	15-16%

In the same row, means with the same letter are not statistically different at the 5% level.

Table 2. Average loads of purple flour samples in CFU/g

	Minimum	Maximum	MC
MAB	2,7±2,5 x 10^{5b}	6,7±2,2 x 10^{5a}	2x10^5-5x10^5
S. aureus	4,5 ±1,1 x10^{3b}	5,7±2,4 x 10^{3a}	10^2-10^3
Total coliforms	4,4±1,1 x 10^{3b}	5,8±1,5 x 10^{3a}	10^2-10^3
Thermotolerant coliforms	1,5±0,7 x10^{3b}	3,6±1,4 x 10^{3a}	10^2
Mold	2,1±0,6 x 10^{4b}	5,0±0,5 x10^{4ab}	10^4-5x10^4
E. coli	ND	ND	10
Anaerobic sulfite-reducing bacteria	ND	ND	100

MC : Microbiological criteria, MAB : Mesophilic aerobic bacteria, ND : Not detected. In the same row, means with the same letter are not statistically different at the 5% level.

Table 3. Percentage of occurrence of isolated fungal species

	Aspergillus Sp1	*Aspergillus* Sp2	*Aspergillus* Sp3
Purple corn flour	29	32	39

Microbial load of analyzed samples

The results of the microbiological analyses focused on counting and testing for MAB, *Staphylococcus aureus*, total and thermotolerant coliforms, *E. coli*, ASR, and molds (Table 2). These microorganisms are indicators used to evaluate the sanitary and hygienic qualities of flour. In general, all germs of hygienic interest were present in all samples. The GAM load ranged from 2.7 to 6.7 × 10^5 CFU/g. Contamination levels for total and thermotolerant coliforms were 4.4 to 5.8 × 10^3 and 1.5 to 3.6 × 10^3 CFU/g, respectively. *Staphylococcus aureus* contamination levels in the samples ranged from 4.5 to 5.7 × 10^3 CFU/g. All of these contamination levels were above the thresholds set by the standards. The average mold load ranged from 2.1 to 5 × 10^4 CFU/g. All samples complied with the microbiological criterion set by the standard (10^4-10^5 CFU/g). Anaerobic sulfite-reducing bacteria (ASR) and *E. coli* were not detected in the analyzed samples.

Presumptive identification of isolated molds

Based on the macroscopic and microscopic characteristics observed, as well as the identification keys provided by Bendjoudi and Dehimi (2020), three species of *Aspergillus* were presumptively identified in the analyzed flours. These species are *Aspergillus*. Sp1, *Aspergillus*. Sp2, and *Aspergillus*. Sp3 (Fig. 2). The occurrence percentages were 29%, 32%, and 39% for *Aspergillus*. Sp1, *Aspergillus*. Sp2, and *Aspergillus*. Sp3, respectively (Table 3).

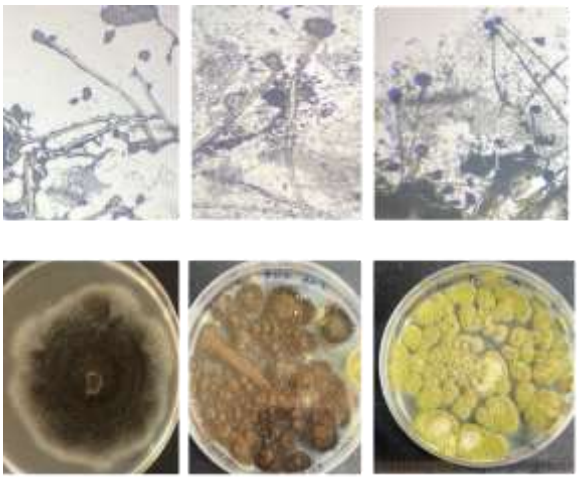

Fig. 2. Macroscopic and microscopic observations of molds

DISCUSSION

Titratable acidity and pH provide an estimate of the amount of acid present in flour, which enables an

assessment of its quality. These parameters can influence flour's behavior during food preparation. They give an idea of the freshness of the flour. The pH and titratable acidity values of the corn flour samples in our study are similar to the values reported by Sika *et al.* (2019) for acidic corn flours. These values ranged from a pH of 5.12 to 6.75 and a titratable acidity of 0.34% to 0.51%. The acidic pH of flours reduces the activity of certain microorganisms.

The moisture level in the flour is below standard (15-16%), which indicates that the flour has been well preserved and limits mold growth. The relatively low moisture level could be due to the flour being properly dried. The Katiola region is sunny, with temperatures often exceeding 37°C.The results obtained in our study are lower than those reported by N'Goran-Aw *et al.* (2018). These authors obtained moisture contents ranging from 21.0% to 39.7% for white corn flour and from 28.2% to 39.8% for yellow corn flour. They attribute these moisture levels to the grains being soaked for hours, or even a whole day, before being processed into flour, as well as to inefficient drying (N'Goran-Aw *et al.*, 2018). High moisture content in flour can promote spoilage during storage. There is a proven relationship between food moisture content and microbial growth capacity. Moisture promotes the proliferation of microorganisms that can hydrolyze starch with their amylases, facilitating the acidification of flour (Houssou *et al.*, 2016a; N'Goran-Aw *et al.*, 2018). Sujitha *et al.* (2018) states that the water content, an important parameter, should generally be between 13% and 15% for proper flour preservation. When flour needs to be stored, it must be dried further in the sun or shade to extend its shelf life.

The results of the microbiological analysis indicate that the level of contamination by MAB, *Staphylococcus aureus*, total coliforms, and thermotolerant coliforms exceeds the threshold set by standards. The presence of these bacteria and the level of contamination suggest that the samples are of poor hygienic quality. This could be due to unsuitable processing, storage, or selling methods. These microorganisms indicate the hygiene conditions of the food manufacturing process (Sousa, 2008 ; Sika *et al.*, 2019). The milling and sun-drying stages, as well as sanitation levels at production and sales sites, are critical points (Adjiléa *et al.*, 2015; Houssou *et al.*, 2016a). A similar study by N'Goran-AW *et al.* (2018) found maximum contamination levels of 1.8×10^9 ; 1.3×10^6 ; 7×10^5 ; 1.1×10^6 ; 5.6×10^6, and 1.9×10^8 CFU/g for MAB, total coliforms, thermotolerant coliforms, yeasts, molds, and enterococci, respectively. In terms of MAB contamination, our results are lower but similar in magnitude to those for other germs. However, they are higher than the results obtained by Traore *et al.* (2025) in their study on fonio flour. Their results for FTAM, Staphylococcus spp., and coliforms were 5.1×10^2 CFU/g, 2.9×10^2 CFU/g, and 0.3×10^1 CFU/g, respectively. This difference could be explained by the different nature of the matrices (corn versus fonio).

Neither ASR nor *E. coli* were detected in the analyzed samples. The absence of ASR, in particular, is good news because these bacteria can sporulate and secrete toxins. N'Goran *et al.* (2018) did not detect ASR or *E. coli* in yellow corn flour samples, but they did isolate both bacteria in a few white flour samples. Sanogo *et al.* (2022) did not detect any *E. coli* colonies in a study of local infant flours.

According to the standard threshold (10^4 to 5×10^5 CFU/g), the levels of mold contamination were acceptable. The low moisture content of the analyzed samples limited mold growth.

However, the presence of mold may be due to the storage conditions of the corn flour in multipurpose bags, as well as the flour's exposure without adequate protection during marketing. These factors promote contamination by fungal flora. Our results are similar to those obtained by N'Goran-AW *et al.* (2018) in white and yellow corn flour in Côte d'Ivoire ($10–10^5$ CFU/g). According to these authors, the proliferation of these microorganisms is also favored by the formation of flour residues of various origins in milling machines, as well as by the high humidity of corn kernels. These findings are consistent with those of Manzor *et al.* (2026), who identified temperature, moisture content, gas

composition, and substrate type as factors that promote mold growth. Mold contamination of flour occurs throughout the production chain, from corn harvesting to flour production (Manzor *et al.*, 2026). The three species of fungi identified were all of the genus *Aspergillus*. Tropical regions have environmental conditions that favor fungal proliferation (Carvajal-Moreno, 2022).

Aspergillus, particularly *A. flavus*, contaminates corn in fields (Xu *et al.*, 2026). This fungus can secrete aflatoxin B1. This toxin has been classified as carcinogenic and has been associated with liver cancer and weakened immune systems (Cao *et al.*, 2022). The presence of aflatoxin poses a significant food safety concern.

CONCLUSION

The pH, titratable acidity, and moisture content of the analyzed flours were consistent with the standards. However, microbiological analyses indicated that the hygiene of the flour samples was unsatisfactory. However, the level of fungal contamination was below the standard. However, the *Aspergillus* species isolated from the samples can secrete dangerous toxins. Anaerobic sulfite-reducing bacteria and *E. coli* were not detected in the analyzed samples. Training saleswomen in good hygiene practices would improve the quality of purple corn flour sold in Katiola markets.

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