

# Influence of Soaking Corn Kernels (*Zea mays* L.) with or without potash on the Fungal and Physico-chemical Quality of Their Flour

YAO Konan Mathurin<sup>1,\*</sup>, KOFFI Koffi Marc<sup>2</sup>, KAMBIRE Ollou<sup>1</sup>,  
DORE Guea Carine Esther<sup>2</sup>, Rose KOFFI-NEVRY<sup>2</sup>, BOLI Zamblé Bi Irié Abel<sup>2</sup>

<sup>1</sup>Biochemistry and Genetics Department, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire

<sup>2</sup>Food Sciences and Technology Department, Nangui Abrogoua University, Abidjan, Côte d'Ivoire

\*Corresponding author: [ykmathurin@gmail.com](mailto:ykmathurin@gmail.com)

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**Abstract** Corn is one of the most consumed cereals in Côte d'Ivoire. The objective of this study is to assess the impact of the soaking time of corn kernels with or without potash on the fungal and physicochemical quality of their flour. The method used consisted of determining the pH of the different samples using a pH meter. For the water content, the method used was oven drying. Concerning the fungal load, the mould count was done on the DRBC medium and the subculture on the MEA medium. All these analyses were carried out with flour samples of two corn varieties (yellow and white corn with or without potash) according to three durations (6, 9 and 12 h) of grains soaking. The results obtained show that yellow or white corn flours without potash are acidic with pH values between 4.2 and 6.1 while those with potash are basic, with pH ranging from 7.7 to 9.9. The moisture content of all the flours obtained just after grinding the grains varies between 31.7 and 37.6%. Significant differences were observed between the water contents of the flours obtained at the three different soaking times. Thus, the water contents of the flour obtained after 6 h of soaking the grains are lower than those obtained after 9 and 12 h of soaking. All cornmeal samples analyzed showed high fungal loads ( $10^5$  to  $10^9$  CFU/g). However, a slowdown in fungal growth was observed with the flour samples with potash. The different moulds isolated from corn flour and identified belong to the genera *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. The genera *Aspergillus* and *Penicillium* are potential producers of mycotoxins and therefore capable of causing poisoning in consumers of these flours.

**Keywords:** corn flour, soaking time, potash, fungal quality

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## 1. Introduction

On a global scale, cereals occupy a primordial place in the agricultural system. They are considered a major source of human and animal nutrition in most countries of the world, as they provide the largest share of protein in the diet. Indeed, cereals provide 57% of the protein consumed, compared to 23% provided by tubers and legumes and 20% by animal products [1]. Maize, from the Poaceae family with the binomial name *Zea mays* (L.), is one of the most widely cultivated cereals in the world [2]. Its usefulness within a population is greatly influenced by the economic disposition of that population. In fact, in developed countries, maize is used more in animal feed and is the raw material for processing industries. However, in low-income countries, its use is more for human food than for feed. It is most often used in developing countries

in the form of cob, semolina or flour. Corn flour is used in the preparation of various traditional products such as couscous (*bashi*), mash (*thô*) and porridge (*baka*). In fact, it is the most important form of maize marketing in Côte d'Ivoire [3]. The areas devoted to maize production have increased considerably in the regions of sub-Saharan Africa since 1961 and Côte d'Ivoire is among the top 20 countries that produce 96% of maize [4]. However, there are a number of constraints related to maize production. These include insufficient and erratic rainfall, soil degradation and depletion, abundant weeds, fungal diseases, and high input costs. Harvesting and storage are generally identified stages of grain contamination that can occur through soil, impurities, dust generated at harvest, and possible sources of infection in conveyances and silos. Mould species contaminating cereals belong most often to the genera *Aspergillus*, *Penicillium* and *Fusarium* [5,6]. These molds are responsible for acute or chronic mycotoxicosis, due to their ability to produce

heat-resistant mycotoxins. To obtain corn flour, the kernels are first moistened by soaking them in water to soften them and make them easier to grind. Potash is often added to corn kernels during soaking to soften certain foods such as "tôh" which will be obtained from flour. Adding potash and increasing grain moisture during steeping could significantly influence the growth of molds that produce secondary metabolites that are carcinogenic to the consumer. In Côte d'Ivoire, particularly in Abidjan markets, from one corn flour seller to another, the duration of grains soaking varies. In order to help reduce health risks due to corn flour consumption, this study aims to assess the influence of the soaking time of corn

kernels with or without potash on the fungal and physico-chemical quality of their flour.

## 2. Material and Methods

### 2.1. Material

The study material consists on the one hand of shelled grains of yellow *Zea mays* and on the other hand of shelled grains of white *Zea mays*, obtained according to the producers' traditional manufacturing process (Figure 1).



**Figure 1.** Photograph of the shelled kernels and corn flour (**A1 and A2:** Yellow corn grains and flour, **B1 and B2:** White corn grains and flour)

## 2.2. Methods

### 2.2.1. Sampling

The sampling consisted of taking two varieties of ordinary corn (yellow and white corn) from a trader at the Lubafrique market in Yopougon-Nianghon (Abidjan/Côte d'Ivoire). Three samples were taken over a three-month period (September to November 2019) at the rate of one sample per month. For each of the two corn varieties, 9 kg of husked corn kernels were collected per month for analysis. In addition, potash was also obtained from the same trader for the processing of some samples.

### 2.2.2. Corn Kernels Processing

Corn flour production is carried out according to the method of [7]. The husked corn kernels were then winnowed and sorted to remove plant debris, stones and other foreign matter. The sorted grains were washed three

times with tap water. After washing, each variety weighing about 9 kg was divided into two large equal parts, each weighing 4.5 kg. One part (4.5 kg) is the corn without potash and the other part (4.5 kg) is the corn with potash added (50 g of potash for 1.5 kg of husked maize kernels) during soaking. According to a survey of farmer-sellers, during the manufacture of corn flour, the soaking time of the kernels after shelling is about 9 hours before milling. Thus, for this study, three soaking times 6 h, 9 h and 12 h were chosen. The 4.5 kg of potash-free yellow corn kernels were subdivided into 3 lots of 1.5 kg each. The first batch was soaked for 12 h, the second for 9 h and the third for 6 h. The 3 batches were soaked separately according to the 3 soaking times indicated above, in 3 different basins each containing 3 L of water, so that the grains are completely submerged. Likewise, the other 4.5 kg of shelled yellow corn kernels were split into 3 lots of 1.5 kg each. Then each batch was soaked in a basin

containing 3 L of water so that it was completely submerged. The first batch was soaked with 50 g of potash for 12 h, the second with 50 g of potash for 9 h and the third batch with 50 g of potash for 6 h. Potash was added to each batch at the start of its soaking. White corn without potash and white corn with potash have also undergone the same treatment.

### 2.2.3. Corn Flour Production

To obtain the flour, after the corn kernels soaking time, the 6 batches (3 batches of yellow corn kernels without potash and 3 batches of yellow corn with potash) were removed from the water at the same time, then drained using 6 sieves 500  $\mu\text{m}$  in diameter (1 sieve for a batch). The drained grains were crushed using a grinder separately to obtain flour and then sieved using 6 different sieves (mesh: 250  $\mu\text{m}$  in diameter). For each batch, 400 g of corn flour were taken and packaged in a stomachers bag, labeled, sealed and then kept in a cooler. The samples were transported to the laboratory on the same day of collection for analyzes. The remaining quantities of the 6 batches of flour were dried in 6 different trays, in the sun for 2 days. After the 2 days of drying, 400 g per batch of flour were taken, packaged individually in stomachers bags, labeled, sealed and then kept in a cooler. The samples were transported to the laboratory on the same day of collection and then they were analyzed. The 6 remaining batches of flour obtained after 2 days of drying were packaged in stomacher bags, sealed and then stored for 5 days, then they were analyzed. A total of 18 samples of approximately 400 g of yellow corn flour were obtained including 9 samples of yellow corn flour without potash and 9 samples of yellow corn flour with potash. This process applies to both white corn kernels without potash and white corn kernels with potash. Thus, for the two varieties of maize, 36 corn flour samples were obtained and analyzed per month. The analyzes were carried out three times under the same conditions over three months. Finally, 108 samples of approximately 400 g of corn flour were obtained at the end of the sampling process.

### 2.2.4. Physico-chemical Analyzes

#### 2.2.4.1. Determination of the pH of Corn Flour

The pH of samples of white and yellow corn flour with or without potash was determined using a pH meter. To do this, the pH was determined by homogenizing 10 g of each flour sample with 100 mL of distilled water. After filtering the solution, the pH value was recorded by inserting the electrode of the pH meter into the filtered product solution and the digital pH value was read on the screen. For each sample, 3 tests were carried out [8].

#### 2.2.4.2. Moisture Content of Corn Flour

The method used is drying in an oven. In fact, 5 g (me) of sample are introduced into a crucible of mass mc. Then, the crucibles containing the samples are placed in an oven for 1 hour at 105°C. After cooling the samples, they are reweighed (ms). This process is repeated until a constant mass is obtained [4]. Three tests are carried out for each sample. The water content of the different samples is determined by the following formula:

$$WC(\%) = \frac{(me + mc) - ms}{me} \times 100 \quad (1)$$

- WC: water content
- me: mass of fresh sample taken
- mc: mass of crucible
- ms: mass of dried sample and crucible

### 2.2.5. Fungal Analysis

#### 2.2.5.1. Mould Count

Ten grams of each corn flour sample was added to 90 mL of sterile buffered peptone water (BPW) in a Stomacher bag (Gosselin, France). The mixture was blended for homogenization for 2 minutes. The suspension obtained was considered as the stock suspension. Subsequently, successive dilutions were made from  $10^{-2}$  to  $10^{-7}$  in 9 mL of sterile buffered peptone water. To estimate the mould population, the surface inoculation method was used. This method allows moulds to have the same access to oxygen and uniform colony development. For this purpose 0.1 mL of each dilution was spread uniformly on DRBC agar, and for each dilution 2 Petri dishes were inoculated. The Petri plates were then incubated at 30°C for 72 h. The colonies were counted then the results were expressed in colony forming unit (CFU) per gram of corn flour. The standard formula [9] used to calculate the fungal load of the different samples is as follows:

$$N = \frac{\sum C}{(n1 + 0,1n2) \cdot d \cdot v} \quad (2)$$

- N: number of microorganisms in CFU / g of corn flour
- $\sum C$ : sum of the colonies counted on the Petri dishes retained at the level of two successive dilutions
- n1: number of boxes retained at the first dilution considered
- n2: number of dishes retained at the second dilution considered
- d: dilution from which the first counts are obtained
- v: volume of inoculum taken (0.1 mL).

#### 2.2.5.2. Subculture Technique

The subculture of a colony on a specific medium contained in a Petri dish makes it possible to obtain a pure culture. This purification is necessary for the identification of moulds. The subculture consists in taking from a colony isolated on a Petri dish, a fragment of mould using tweezers previously sterilized with a benzene beak and then put it on a sterile malt extract medium (MEA) and agar. The new preparation thus obtained is incubated at 30°C for 5 to 7 days.

#### 2.2.5.3. Identification of Isolated Mould Strains

The identification of isolated moulds is made on the basis of the keys published by David Malloch translated and adapted by [10]. These keys made it possible to determine the genus of each strain by means of their microscopic and macroscopic characteristics (appearance, color of the underside of the colonies). For microscopic observation, a portion of the colony was removed and then homogenized in a drop of methylene blue deposited beforehand on a clean slide. The preparation was then covered with a coverslip and observed under an optical microscope such as Optika Microscopes at the X 40 objective.

### 2.2.6. Statistical Analyzes

The results obtained (pH, water content and fungal load of the corn flour samples) were subjected to analysis of variance (ANOVA) with Statistica version 7.1 software. In the event of a significant difference between the parameters studied, the means are classified according to the Newmann-Keuls test. The significance level is  $\alpha = 0.05$ .

## 3. Results and Discussion

### 3.1. Results

#### 3.1.1. pH of Yellow Corn Flour

The pH of the yellow corn flours without potash and of the yellow flours with potash are given in [Table 1](#). Regarding the yellow flour without potash, the results indicate average pH values between 4.2 and 6.1. Statistical analyzes reveal that for each category of flour (F1 = Corn flour obtained just after grinding, F2 = Corn flour dried for 2 days in the sun, F3 = Corn flour kept for 5 days after 2 days of solar drying), there is no significant difference ( $P > 0.05$ ) between the pH of the flours obtained regardless of the soaking time of the corn kernels. The flour of yellow corn with potash, for the most part has a pH varying from 6.3 to 9.1. For each category of corn flour (F1, F2, F3), the pH of the flour does not vary significantly ( $P > 0.05$ ) from one soaking period of the corn kernels to another.

#### 3.1.2. pH of White Corn Flour

White corn flours without potash are acidic and have a pH between 4.2 and 5.2. For each category of white corn flour (F1, F2, F3), the pH of the flour does not vary significantly ( $P > 0.05$ ) from one soaking period of the corn kernels to another. For white corn flours with potash, the average pH values are between 4.5 and 9.9. In general, the average pH values of flour samples with potash are higher than those of flours without potash ([Table 2](#)).

#### 3.1.3. Moisture Content of Yellow Corn Flour

Moisture contents of yellow flours without potash and yellow flours with potash as a function of corn kernel soaking time are given in [Table 3](#). For yellow corn without potash, the average values for the moisture content of the flours (F1) obtained just after milling ranged from 32.8 to 36.7%. Statistical analyses show that there is a significant difference ( $P < 0.05$ ) between the moisture contents of the flours obtained at 6, 9 and 12 h of corn kernel soaking. Drying the flours for 2 days resulted in a decrease in the moisture content of the individual flours from 32.8 to 19.6% for yellow corn flour without potash whose grains have been soaked in water for 6 hours. The results for yellow corn flour with potash were similar to those for flour without potash, with values ranging from 19.8 to 36.7%.

#### 3.1.4. Moisture Content of White Corn Flour

The moisture contents of white corn flours with and without potash in [Table 4](#) show that the flours obtained just after milling the grains soaked in water for 6, 9 and 12 h are significantly different ( $P < 0.05$ ). These values range

from 34.8 to 31.7% for white corn flours without potash and 34.4 to 37.6% for white corn flours with potash. Drying the flours for 2 days resulted in a decrease in the moisture content of the different flours, from 37.6 to 25.1% for white corn flour with potash whose kernels were soaked in water for 12 hours.

#### 3.1.5. Fungal Load of Yellow Corn Flours

[Table 5](#) shows the mould load of the yellow corn flour without potash and that of the yellow corn flour with potash as a function of the soaking time of the kernels. Regarding yellow corn without potash, the flours obtained just after grinding the grains have a mould load of around  $10^5$  CFU / g. The fungal load of the flour obtained after 12 h of soaking the corn kernels recorded the highest mould load ( $6.4 \cdot 10^5$  CFU / g) while that obtained after 6 h of soaking the kernels had the lowest number of moulds. ( $3.8 \cdot 10^5$  CFU / g). After 2 days of drying, fungal growth reached about  $10^6$  CFU/g in all samples. After 5 days of storage, the number of moulds increased considerably in the different flours and ranged from  $1.1 \cdot 10^9$  to  $1.7 \cdot 10^9$  CFU/g. Similarly, for yellow corn with potash, the mould load in the flours obtained just after the milling of the grains is about  $10^5$  CFU/g. After 2 days of drying the flours, the number of moulds increased in the three different flour samples, but did not exceed  $10^5$  CFU/g. After 5 days of storage, the flours dried for 2 days showed fungal loads ranging from  $7.8 \cdot 10^8$  to  $1.3 \cdot 10^9$  CFU/g. All samples showed fungal loads above the microbiological criterion of  $10^3$  CFU/g at most. The longer the soaking time, the greater the fungal contamination.

#### 3.1.6. Fungal Load of White Corn Flour

The mould loads for white corn flours without potash and those for white corn flours with potash are reported in [Table 6](#). Regarding the white corn flour without potash, the mould load in the three samples obtained just after the grinding of the grains, varied from  $4.6 \cdot 10^5$  to  $5.6 \cdot 10^5$  CFU/g. After 2 days of flour drying, the mould load increased in the three different flour samples without exceeding the order of  $10^5$  CFU/g. Flours dried for 2 days and then stored for 5 days saw a significant increase in the number of molds, from  $10^5$  to  $10^9$  CFU/g. For the samples of white corn flour with potash, the flours obtained just after milling have a mould load of about  $10^5$  CFU/g. The flour obtained after 12 h soaking the corn grains recorded the highest mould count ( $5.7 \cdot 10^5$  CFU/g) while the flour obtained after 6 h soaking the grains had the lowest mould count ( $3.8 \cdot 10^5$  CFU/g). After 2 days of flour drying, the number of moulds underwent a slight increase in the three different flour samples. Thus, the fungal load of white corn flour with potash obtained after 6 h of soaking of the grains went from  $3.8 \cdot 10^5$  to  $4.7 \cdot 10^5$  CFU/g after the 2 days of drying, while those obtained after 9 h of soaking increased from  $5.5 \cdot 10^5$  to  $7.1 \cdot 10^5$  CFU/g. In addition, the mould load of the white corn flour with potash obtained after 12 hours soaking varies from  $5.7 \cdot 10^5$  to  $9.4 \cdot 10^5$  CFU/g. After 5 days of storage, the number of moulds increased in the different flours and ranged from  $7.7 \cdot 10^8$  to  $1.4 \cdot 10^9$  CFU/g. All samples showed fungal loadings above the microbiological criterion of  $10^3$  CFU/g at most. In general, the fungal load in flours without potash is higher than in flours with potash.

**Table 1. pH of yellow corn flour with and without potash as a function of the soaking time of the kernels**

Soaking time of corn kernels (h)	pH					
	Potash free flour			Flour with potash		
	F1	F2	F3	F1	F2	F3
6	4.8±0.4 <sup>a</sup>	4.8±0.3 <sup>b</sup>	6.1±0.1 <sup>c</sup>	9.1±0.2 <sup>a</sup>	7.1±0.2 <sup>b</sup>	8.2±0.5 <sup>d</sup>
9	4.9±0.5 <sup>a</sup>	4.2±0.3 <sup>b</sup>	5.5±0.5 <sup>c</sup>	8.6±0.4 <sup>a</sup>	6.3±0.6 <sup>b</sup>	6.4±0.6 <sup>c</sup>
12	4.6±0.1 <sup>a</sup>	4.3±0.3 <sup>b</sup>	5.4±0.5 <sup>c</sup>	8.9±0.9 <sup>a</sup>	6.9±0.3 <sup>b</sup>	7.3±0.4 <sup>cd</sup>

F1 = Corn flour obtained just after milling

F2 = Corn flour dried for 2 days in the sun

F3 = Corn flour stored for 5 days after 2 days of solar drying

In column, the means assigned the same letter are not significantly different at the 5% level according to the Newmann-Keuls test.

**Table 2. pH of white corn flour with and without potash as a function of the soaking time of the kernels**

Soaking time of corn kernels (h)	pH					
	Potash free flour			Flour with potash		
	F1	F2	F3	F1	F2	F3
6	4.6±0.3 <sup>a</sup>	4.5±0.1 <sup>b</sup>	5.2±0.1 <sup>c</sup>	9.9±0.8 <sup>b</sup>	7.6±0.6 <sup>d</sup>	5.7±0.6 <sup>e</sup>
9	4.4±0.1 <sup>a</sup>	4.3±0.2 <sup>b</sup>	4.9±0.4 <sup>c</sup>	8.4±0.9 <sup>ab</sup>	6.3±0.8 <sup>c</sup>	5.8±0.3 <sup>e</sup>
12	4.2±0.3 <sup>a</sup>	4.3±0.1 <sup>b</sup>	5.2±0.1 <sup>c</sup>	7.7±0.6 <sup>a</sup>	6.1±0.1 <sup>c</sup>	4.5±0.4 <sup>f</sup>

F1 = Corn flour obtained just after milling

F2 = Corn flour dried for 2 days in the sun

F3 = Corn flour stored for 5 days after 2 days of solar drying

In column, the means assigned the same letter are not significantly different at the 5% level according to the Newmann-Keuls test.

**Table 3. Moisture content of yellow corn flour with and without potash as a function of the soaking time of the kernels**

Soaking time of corn kernels (h)	Moisture content (%)					
	Potash free flour			Flour with potash		
	F1	F2	F3	F1	F2	F3
6	32.8±1.3 <sup>a</sup>	19.6±1.5 <sup>d</sup>	18.3±1.2 <sup>f</sup>	33.1±1.9 <sup>a</sup>	21.3±1.1 <sup>c</sup>	19.8±0.9 <sup>d</sup>
9	35.0±0.8 <sup>b</sup>	21.3±1.4 <sup>d</sup>	19.8±1.1 <sup>f</sup>	34.8±1.4 <sup>a</sup>	23.6±0.5 <sup>b</sup>	21.9±0.6 <sup>e</sup>
12	36.7±0.3 <sup>c</sup>	26.2±0.7 <sup>e</sup>	20.5±1.4 <sup>f</sup>	36.7±0.9 <sup>a</sup>	24.5±0.7 <sup>b</sup>	24.1±0.2 <sup>f</sup>

F1 = Corn flour obtained just after milling

F2 = Corn flour dried for 2 days in the sun

F3 = Corn flour stored for 5 days after 2 days of solar drying

In column, the means assigned the same letter are not significantly different at the 5% level according to the Newmann-Keuls test.

**Table 4. Moisture content of white corn flour with and without potash as a function of the soaking time of the kernels**

Soaking time of corn kernels (h)	Moisture content (%)					
	Potash free flour			Flour with potash		
	F1	F2	F3	F1	F2	F3
6	31.7±0.2 <sup>a</sup>	20.5±0.4 <sup>c</sup>	19.4±0.5 <sup>f</sup>	34.4±0.6 <sup>a</sup>	20.7±0.7 <sup>d</sup>	19.7±0.8 <sup>f</sup>
9	34.8±0.7 <sup>b</sup>	23.4±0.6 <sup>d</sup>	21.2±1.1 <sup>e</sup>	36.2±0.2 <sup>b</sup>	22.1±0.9 <sup>d</sup>	20.0±0.9 <sup>f</sup>
12	36.6±0.4 <sup>c</sup>	24.7±1.1 <sup>d</sup>	22.8±0.5 <sup>b</sup>	37.6±0.7 <sup>c</sup>	25.1±0.9 <sup>e</sup>	22.2±1.1 <sup>e</sup>

F1 = Corn flour obtained just after milling

F2 = Corn flour dried for 2 days in the sun

F3 = Corn flour stored for 5 days after 2 days of solar drying

In column, the means assigned the same letter are not significantly different at the 5% level according to the Newmann-Keuls test.

**Table 5. Effect of soaking time of yellow corn kernels with and without potash on the fungal load of their flour**

Soaking time of corn kernels (h)	Mould count (CFU/g)					
	Potash free flour			Flour with potash		
	F1	F2	F3	F1	F2	F3
6	3.8±1.1x10 <sup>5a</sup>	1.1±0.7x10 <sup>6</sup>	1.6±0.1x10 <sup>9d</sup>	4.2±0.7x10 <sup>5a</sup>	4.8±1.2x10 <sup>5b</sup>	7.8±0.9x10 <sup>8d</sup>
9	4.4±0.5x10 <sup>5a</sup>	1.2±0.2x10 <sup>6c</sup>	1.3±0.1x10 <sup>9d</sup>	4.8±0.1x10 <sup>5a</sup>	6.8±0.6x10 <sup>5bc</sup>	9.4±0.1x10 <sup>8e</sup>
12	6.4±1.4x10 <sup>5b</sup>	1.3±0.3x10 <sup>6c</sup>	1.7±0.3x10 <sup>9d</sup>	5.5±0.3x10 <sup>5a</sup>	8.9±1.4x10 <sup>5c</sup>	1.3±0.1x10 <sup>9f</sup>
Microbiological criterion	10 <sup>3</sup> UFC/g			10 <sup>3</sup> UFC/g		
Microbiological standard	ISO 21527-1			ISO 21527-1		

F1 = Corn flour obtained just after milling

F2 = Corn flour dried for 2 days in the sun

F3 = Corn flour stored for 5 days after 2 days of solar drying

In column, the means assigned the same letter are not significantly different at the 5% level according to the Newmann-Keuls test.

**Table 6. Effect of soaking time of white corn kernels with and without potash on the fungal load of their flour**

Soaking time of corn kernels (h)	Mould count (CFU/g)					
	Potash free flour			Flour with potash		
	F1	F2	F3	F1	F2	F3
6	4.6±0.2x10 <sup>5a</sup>	6.7±0.5x10 <sup>5b</sup>	7.9±0.3x10 <sup>8d</sup>	3.8±0.6x10 <sup>5a</sup>	4.7±0.2x10 <sup>5c</sup>	7.7±0.6x10 <sup>8e</sup>
9	4.9±0.4x10 <sup>5a</sup>	8.3±1.1x10 <sup>5c</sup>	1.1±0.2x10 <sup>9e</sup>	5.5±0.1x10 <sup>5b</sup>	7.1±1.3x10 <sup>5d</sup>	9.4±1.2x10 <sup>8e</sup>
12	5.6±1.2x10 <sup>5b</sup>	9.6±0.8x10 <sup>5c</sup>	1.9±0.1x10 <sup>9e</sup>	5.7±1.2x10 <sup>5b</sup>	9.4±1.1x10 <sup>5d</sup>	1.4±0.1x10 <sup>9f</sup>
Microbiological criterion	10 <sup>3</sup> UFC/g			10 <sup>3</sup> UFC/g		
Microbiological standard	ISO 21527-1			ISO 21527-1		

F1 = Corn flour obtained just after milling

F2 = Corn flour dried for 2 days in the sun

F3 = Corn flour stored for 5 days after 2 days of solar drying

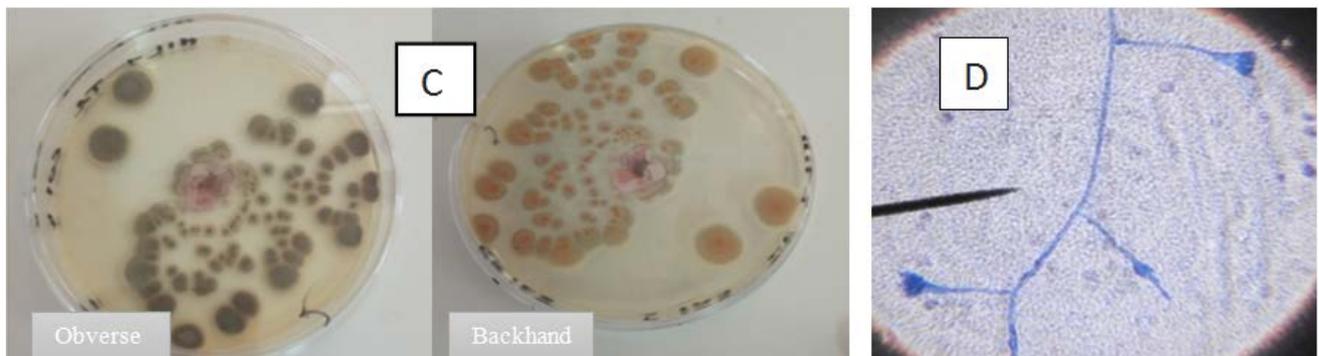
In column, the means assigned the same letter are not significantly different at the 5% level according to the Newmann-Keuls test.

### 3.1.7. Microscopic and Macroscopic Characteristics of Isolated Fungi

Analysis of the natural mycoflora of the various corn flour samples revealed the presence of the genera *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus* (Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6). This identification was based on macro and microscopic analysis of fungal isolates on malt extract medium (MEA).



**Figure 2.** *Aspergillus* sp 1. Macroscopic aspect (A) and microscopic aspect (B)



**Figure 3.** *Penicillium* sp. Macroscopic aspect (C) and microscopic aspect (D)



**Figure 4.** *Mucor* sp. Macroscopic aspect (E) and microscopic aspect (F)



Figure 5. *Aspergillus* sp 2. Macroscopic aspect (G) and microscopic aspect (H)



Figure 6. *Rhizopus* sp. Macroscopic aspect (I) and microscopic aspect (J)

### 3.2. Discussion

This study focused on the determination of some physicochemical and fungal parameters of yellow and white corn flours with or without potash depending on the soaking time of the kernels.

Irrespective of the soaking time of the grains, the water contents recorded for all the flours tested are very high even after 2 days of drying. The results show that the moisture content of the individual samples ranges from 18.3 to 37.6%. These moisture values are consistent with those obtained by [3] who showed a high moisture content in white and yellow flours with potash marketed in 9 communes of Abidjan. The high moisture content reflects the addition of water when soaking the corn kernels. According to [11], the moisture content, which is an important parameter, is generally between 13 and 15% for good flour preservation. Too much moisture causes flour spoilage. It has been shown that there is a relationship between the moisture in the food and the microbial growth capacity [5]. Thus, according to several studies, the low water content of food products prevents the enzymatic activities of microorganisms from altering and thus prevents the deterioration of food during storage [12]. The high moisture content (above 15%) of the samples that underwent 2 days of drying suggests that the 1-2 day drying usually practiced by cornmeal producers and sellers is insufficient to reduce the moisture content of the flours below 15%. The longer the soaking time, the higher the moisture content, but there is no significant difference ( $P > 0.05$ ) between the moisture content values of the corn flours obtained at the three different grain soaking times.

Potash did not influence the moisture content of the corn flour samples.

Flours without potash are acidic while those with potash are basic. Depending on temperature, humidity and storage time, flours acquire a certain acidity more or less quickly, indicating deterioration for certain acid concentrations [13]. This acidity causes a drop in pH and inhibits the growth of certain bacteria, including Gram-negative bacteria [14], but not mould [15]. The pH of the flours decreases as the soaking time of the grains increases. This result could be explained according to the hypothesis put forward by Sall (1998), that a high water content in flours made on a cottage industry scale promotes starch hydrolysis and the formation of acetic acid. This would lead to a low pH value.

All the corn flour samples studied showed high fungal loads ( $10^5$  to  $10^9$  CFU/g). These levels of contamination are above the microbiological criterion, which is a maximum of  $10^3$  CFU/g. However, in general, the mould load in flour without potash is higher than that in flour with potash. "Toh" is a food obtained from corn flour with potash and recommended for people who have undergone surgery. This result could cause a short-term deterioration of these flours and lead to food poisoning following the formation of mycotoxins as also indicated by [16]. Flours without potash were the most contaminated by moulds because of their acidity. Indeed, moulds thrive best in an acidic environment. These results corroborate those of [17] who showed that the acidity of wheat grains increases fungal growth. Most moulds grow under acidic pH conditions and can tolerate very low pH values [18].

The longer the soaking time, the greater the fungal contamination, which means that although the purpose of

grain soaking is to soften the grains to make them easier to grind, it also causes the fungal quality of the flours to deteriorate. Thus, the high water content is the limiting factor for the preservation of artisanally produced corn flour. There are many sources of contamination, but in the case of traditional mills, the proliferation of germs is due to the formation of flour residues of various origins in the milling machines and the high moisture content of the maize grains. Hygienic conditions in mills are often factors of cross-contamination. Indeed, artisanal mills are used for the production of several different products without cleaning after each grinding as mentioned by [19] and [20]. The different genera of moulds isolated are *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. This result is similar to that of [4] with the exception of *Botrytis* and *Fusarium*. These moulds are likely to produce mycotoxins which are secondary metabolites with low labile and often active at very low doses. They diffuse into the substrate which they contaminate even after the destruction of the fungus responsible for their production.

#### 4. Conclusion

Corn flour is a much appreciated food product but the artisanal conditions of its production make it a potential source of poisoning for the consumer. The objective of this work was to evaluate the influence of the soaking time of corn kernels with or without potash on the fungal and physico-chemical quality of their flour. This study showed that corn flour without potash is acidic while that with potash is basic. The basicity of the flour slows down fungal growth. The study also showed that the level of contamination in cornmeal is proportional to the increase in soaking time and that potash influences mould growth. The different moulds isolated and identified belong to the genera *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. The genera *Aspergillus* and *Penicillium* are potential producers of mycotoxins. Thus, consuming corn flour containing these toxigenic strains represents a real danger for consumers. Beyond the impact of the soaking time of the grains on the fungal quality of the corn flour, the artisanal milling conditions of the grains, which do not respect any hygienic measures, constitute an essential link in the chain of contamination of the corn flour.

#### Statement of Competing Interests

The authors have no competing interest in relation to their work.

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