Evaluation of the Allergenic Potential of Anagobaka infant Flours Marketed in the Markets of the District of Abidjan

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Authors’ contributions

This work was carried out in collaboration among all authors. Author CCAYC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SEK and AB the analyses of the study. Author IB managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

The aim is to evaluate the sanitary quality of the infant flours of "Anagobaka" imported and sold on the markets of the district of Abidjan. There are eleven flours of "Anagobaka" distributed as follows, four from Adjame, four from Abobo and three from Treichville, a local artisanal infant flour. An
1. INTRODUCTION

The problem of food security around the world includes the health hazard, related to the toxic-infections, pests and food allergens in the vast majority. Cases of microbiological, chemical and physical risks but also allergic risks can be caused by consumption of animal and vegetable foods. There is more and more scientific data shedding light on the nature and significance of these risks, although some information-gathering tools such as monitoring need to be strengthened [1]. Deeply concerned by this problem, WHO Member States adopted in 2000 a resolution recognizing that food safety related to hygiene, safety and food safety is an essential aspect of public health. These are diseases that can be transmitted to humans through the consumption of food contaminated with pathogens in water, soil or air. According to the WHO, every year around the world, 600 million people suffer from foodborne illness and 420,000 people die, including 125,000 children under five, after consuming contaminated food. It is estimated that Africa has the highest burden of foodborne diseases per population. More than 91 million people become ill and 137,000 die each year [2].

At present, they constitute one of the most widespread public health problems on an international scale. Policies and measures in this area must provide as safe food as possible, taking into account the entire food chain, from production to consumption [3]. Particularly susceptible populations are young children, the elderly, pregnant women and people with immunocompromised systems. The official sanitary control services record a dominance of germs such as Salmonella, Sulfito-Reducer Anaerobes, Clostridium, Staphylococcus, Coliforms, yeasts and molds in foodstuffs subject to consumption in Côte d’Ivoire [4]. Some germs and chemicals escape this control: parasites, virus, mycotoxins, allergens, pesticide residues, heavy metals and synthetic additives. Among the plant foods most commonly contaminated by molds are foods based cereals and oil seeds. These molds can secrete mycotoxins which are toxic metabolites whose ingestion causes intoxication in the consumer providing that their concentration is sufficiently high to produce an effect and are recognized for their mutagenic, carcinogenic and teratogenic properties [5]. In Côte d’Ivoire, as elsewhere in Africa, weaning flours are used as nutritional supplements for children’s diets, in the context of food diversification. During this weaning period, mothers usually feed their children with traditional porridges made from simple or compound flours from cereals and tubers that are high in carbohydrate foods but low in protein [6,7]. These foods can not cover all the nutritional needs of the child [8,9]. The quality of the infant flours used during this period is therefore of great importance. Local or imported industrial infantile flours are of better nutritional quality but remain inaccessible to the majority of households with low purchasing power. These mothers get their supplies from distributors, who are Nigerian traders, from cheap industrial flour. Mothers as well as these traders have no knowledge of the nutritional and sanitary quality of these products. These traders provide infant flours made in Nigeria from the Custard Powder brand in all districts of the District of Abidjan, hence the name "Anagobaka". Anago is the term commonly used in Côte d’Ivoire to designate people from Nigeria, while the cereal porridge is locally called "Baka". "Anagobaka" flours are fine-textured industrial infant flour has been used as a reference. Moisture and pH were measured by the Association of Official Analytical Chemists and contaminants by microbiological methods. Moisture contents of "Anagobaka" flours ranged from 8.79% ± 0.06 to 12.94% ± 0.49. That of local flours was 6.56% ± 0.21. These values are above the norm of 5%, while the reference has a content which is 3.59% ± 0.2 respecting the norm. However, all these flours have an acidic pH of 5.25 ± 0.01 at 6.83 ± 0.01 After inoculation on Sabouraud chloramphenicol medium with the various suspensions of flours, the species Aspergillus flavus was identified in the brands, Jone Family, Bolero strawberry, Lady B, Fincap, Egg Banana and Glad Family at respective loads of 1400 cfu / g ± 173.21, 11.10\(^2\) cfu / g ± 200, 9.10\(^2\) cfu / g ± 200, 6.10\(^2\) cfu / g ± 100, 4.10\(^2\) cfu / g ± 100 and 2.10\(^2\) cfu / g ± 00. (Whereas, the association Aspergillus flavus and Mucor sp was found in the Jone Family brand flour, at a load of 14.10\(^2\) cfu / g ± 173.21.) All flours were free of other germs. The presence of molds does not meet microbiological standards recommended and represent a danger to the health of these children.

Keywords: Aspergillus flavus; Mucor sp; contaminants; infant flours; Abidjan.
Cream powders based on corn starch in which, salt, flavoring, coloring, vitamins and minerals are added with or without the addition of egg yolk [10,11]. They are mainly high in carbohydrates, low in protein and do not meet Codex standards established by FAO/WHO [12].

They would expose the infant population to malnutrition, which is often due to the consumption of flours, which are low in protein, micronutrients and unbalanced in energy responsible for long-term severe metabolic insufficiency [13,14,15,16]. It is therefore essential to ensure the safety of all over-the-counter (OTC) food flour in the markets of our developing countries, knowing that the techniques for determining the microbiological parameters of foods are efficient, accessible and easy to put into practice implemented. In addition, no studies have been carried out to date on certain microbiological food contaminants in infantile "Anagobaka" flours marketed in Côte d'Ivoire. In order to contribute to the food security of children at the time of weaning, various brands of "Anagobaka" infant flours, marketed in local markets of the Abidjan district, were evaluated for their microbiological and physicochemical properties.

2. MATERIALS AND METHODS

2.1 Technical Materials

It consists of an oven (Heraeus), an oven (MEMMERT, 854 shwabach), pH meter (HANNA INSTRUMENTS, HI 8424), a CO₂ incubator (P Selecta®, 100% CO₂, 100°C), an optical microscope (Micromaster®, Fisher Scientific), a precision balance (sartorius), a hot plate, a refrigerator, an autoclave for sterilizing equipment.

The samples of weaning flour of different commercial brands collected were coded as follows 1st letter = (ADJ) name of the commune, 1st digit = (001) No of the sample and 2nd digit (18) = the year of sampling (Table 1).

2.2 Sample Collection

For this study, eleven samples of Anagobaka infantile flours of different brands were purchased from traders in markets in the District of Abidjan. A sample of local artisanal maize flour and a sample of Cerelac rice flour used as a reference were purchased at a supermarket in Cocody commune. All the flour samples purchased had expiry dates of 2019 and there was no physical damage observed on the polyethylene packaging. Then they were sent to the Pasteur Institute of Côte d’Ivoire (IPCI). Then these flours were taken from sterile coproculture plates with sterile spoon around the flame in a sterile area and stored at room temperature for microbiological and physicochemical analysis.

2.3 Physico-chemical Analyzes

2.3.1 Determination of moisture content

The moisture content of infant flours was determined by the AOAC 925.10 method [17]. To do this, the vacuum capsule was first cleaned, dried and weighed (m₀). A quantity of infant flours (5 g) was introduced into the well-weighed vacuum capsule (m₁ = m₀ + 5 g) and the assembly was placed in the Heraeus oven at 105°C for 24 hours. After drying, the capsule was removed from the oven, cooled in a desiccator (P₂O₅) and weighed (m₂) again. The moisture content (%) was determined by the following formula:

\[
H(\%) = \frac{(m₁ - m₂) \times 100}{(m₁ - m₀)}
\]

2.3.2 pH measurement

This measurement was done according to the method (AOAC, 1990) [17]. Ten (10 g) of sample (me) were homogenized in 100 ml of distilled water by stirring the mixture in a beaker. The mixture is then filtered through filter paper (Whatman) and the filtrate is collected in an Erlenmeyer flask. The reading of the pH is carried out directly on the filtrate after calibration of the pH-meter by buffer solutions pH 4.00 and pH 7.00.

2.4 Research of Microbiological Contaminants

2.4.1 Preparation of the stock solution and decimal dilutions

As part of the enumeration of sprouts, 25 g of each sample of infant flours were collected with sterile spoon into aseptic Stomachers sachets using an electronic scale and then mixed with 225 ml of sterile buffered peptone water. The mixture is homogenized for 2 min, the suspension obtained corresponds to the stock solution. For solid products the mother
suspension is usually $10^{-1}$ dilution. From this suspension mother, a range of decimal dilutions is carried out. 1 ml of the mother suspension is withdrawn and diluted in 9 ml of tryptone salt, thus obtaining the $10^{-2}$ dilution; the same operation is repeated, starting from the $10^{-2}$ dilution, to obtain the $10^{-3}$ dilution. The operation continues until the dilution $10^{-6}$ is obtained.

2.4.2 Seeding in the mass

1 ml of stock solution and 1 ml of each dilution are inoculated in various specific agaric nutrient media, poured into Petri boxes. The nature of the culture medium used and the incubation conditions depend in fact on the nature of the seeds to be counted.

2.4.2.1 Mesophilic aerobic germs

For Mesophilic Aerobic (Germs) Spread counts, the inocula (inoculum) are seeded in Plate Count Agar (PCA) agar by mass incorporation and a second layer of Pastagar A is poured after cooling. 1 ml of the stock solution and 1 ml of the decimal dilutions are placed in the Petri (boxes) dishes using a sterile pipette. Then 15 ml of PCA agar supercooled. After cooling and solidification, a second layer of 5 ml of Pastagar A is cast onto the PCA agar. The Petri dishes (boxes) are put in an oven at 37°C. for 48 hours. All white colonies will be taken into account.

2.4.2.2 Enterobacteria

For Enterobacteria count, the inoculum are seeded into VRBG agar by incorporation into the mass (double layer). 1 ml of the stock solution and 1 ml of the decimal dilutions are placed in the Petri dishes (boxes) using a sterile pipette. 15 ml of the VRBG agar are then supercooled. After cooling and solidification, a second layer of 5 ml of VRBG is cast on the first layer. Petri boxes are incubated at 30°C for 24 h. All bulging colonies showing a bright red color with diffuse contours will be taken into account.

2.4.2.3 Total and thermotolerant coliforms

For counting total coliforms and thermotolerant coliforms, the inocula (inoculum) are inoculated (seeded) into the VRBL agar by incorporation into the mass (double layer). 1 ml of the stock solution and 1 ml of the decimal dilutions are placed in the petri dishes (box) using a sterile pipette. Then 15 ml of the VRBL agar supercooled. After cooling and solidification, a second layer of 5 ml of VRBL is cast onto the first layer. Petri dishes boxes are incubated at 30°C for CT and 44°C for HTC for 24 h. All red - bright colonies with diffuse contours will be taken into account.

2.4.3 Surface seeding

2.4.3.1 Staphylococci

Staphylococci are isolated on Baird Parker medium supplemented with potassium tellurite and egg yollow. 15 ml of the supplemented Baird Parker agar are poured into the petri dish, then the medium is solidified and oven-dried. 0.1 ml of the stock solution is placed in the Petri boxe, using a sterile spreader. The inoculum is spread over the entire surface and incubated at 37°C for 24 h. All black-bright colonies surrounded by bright halo will be taken into account.

<table>
<thead>
<tr>
<th>N°</th>
<th>Trade name of the analyzed products</th>
<th>Codification of products</th>
<th>Type of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Bolero pineapple</td>
<td>ADJ/001/18</td>
<td>Flours</td>
</tr>
<tr>
<td>02</td>
<td>Fincap</td>
<td>ADJ/003/18</td>
<td>of Anagobaka</td>
</tr>
<tr>
<td>03</td>
<td>Jone Family</td>
<td>ADJ/004/18</td>
<td>(Custard Powder)</td>
</tr>
<tr>
<td>04</td>
<td>Tolex</td>
<td>ADJ/006/18</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>Bolero strawberry</td>
<td>ABO/001/18</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>Egg Banana</td>
<td>ABO/003/18</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>Queen Royale</td>
<td>ABO/005/18</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>Prime</td>
<td>ABO/006/18</td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>Family Milk</td>
<td>TREICH/001/18</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Lady B</td>
<td>TREICH/002/18</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Glad Family</td>
<td>TREICH/003/18</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Corn flour</td>
<td>COCO/001/18</td>
<td>Artisanal flour</td>
</tr>
<tr>
<td>13</td>
<td>Cerelac of rice</td>
<td>COCO/002/18</td>
<td>Industrial flour</td>
</tr>
</tbody>
</table>
Table 2. Moisture content and pH of the various infant flours analyzed

<table>
<thead>
<tr>
<th>Flours.</th>
<th>pH</th>
<th>P-Value</th>
<th>Moisture content</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolero. P</td>
<td>6.60±0.01</td>
<td>&lt;0.0001</td>
<td>11.92±0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fincap</td>
<td>6.83±0.01</td>
<td>&lt;0.0001</td>
<td>12.94±0.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Jone Family</td>
<td>6.63±0.03</td>
<td>&lt;0.0001</td>
<td>11.28±0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tollex</td>
<td>6.82±0.02</td>
<td>0.0008</td>
<td>12.47±0.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bolero Strawberry</td>
<td>6.64±0.01</td>
<td>&lt;0.0001</td>
<td>11.42±0.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Egg Banana</td>
<td>6.82±0.01</td>
<td>0.0019</td>
<td>10.46±0.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Queen Royale</td>
<td>5.94±0.01</td>
<td>&lt;0.0001</td>
<td>12.47±0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prime</td>
<td>6.43±0.02</td>
<td>&lt;0.0001</td>
<td>9.31±0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family Milk</td>
<td>6.32±0.01</td>
<td>&lt;0.0001</td>
<td>8.79±0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lady B</td>
<td>6.67±0.01</td>
<td>&lt;0.0001</td>
<td>12.56±0.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glad Family</td>
<td>6.38±0.01</td>
<td>&lt;0.0001</td>
<td>11.87±0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Farine de Maïs</td>
<td>5.25±0.01</td>
<td>&lt;0.0001</td>
<td>6.56±0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cerelec Riz</td>
<td>6.76±0.01</td>
<td>&lt;0.0001</td>
<td>3.59±0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Standard FAO/OMS 2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.4.3.2 Escherichia coli

*Escherichia coli* are isolated on Rapid *E. coli* 2 (agar). 15 ml of Rapid *E. coli* 2 agar are poured into the Petri boxe, then the medium is solidified and oven-dried. 0.1 ml of the stock solution is dispensed into the Petri boxe, using a sterile spreader. The inoculum is spread over the entire surface and incubated at 44°C for 24 h. All purple to pink and S-type colonies will be considered.

2.4.3.3 Anaerobic sulfito reducers

Sulfito Reducing Anaerobes are isolated on TSN (Tryptone Sulfite Neomycin) agar. 0.1 ml of the stock solution is placed in a screw tube and incubated at 46°C for 24-48 h. All the big black colonies will be taken into account.

2.4.3.4 Yeasts and molds

Yeasts and molds are isolated on Sabouraud. 15 ml of the agar are poured into the petri boxe, then the medium is solidified and dried in an oven. 0.1 ml of the stock solution is dispensed, using a sterile spreader. The inoculum is spread over the entire surface and incubated at 30°C for 48 h. All the white, smooth, creamy colonies of yeasts and molds in powdery, cottony and variable colors will be taken into account.

2.4.3.5 Salmonella

For *Salmonella* research, pre-enrichment is performed from the stock solution at 37°C for 18 to 24 h. Enrichment in a selective medium is carried out in Bouillon Rappaport Vassiliadias. 0.1 ml of the pre-enrichment broth is placed in a screw tube containing 10 ml of Bouillon Rappaport Vassiliadias, the whole is put at 42°C for 24 h. This second step aims to minimize the growth of bacteria other than *salmonella*. For selective isolation, Hektoen Agar is prepared and poured into Petri boxe. Then 10 µl of each selective enrichment culture are seeded on Hektoen agar and incubated at 37°C for 24 h. On Hektoen agar, all greenish colonies with or without a black center will be taken into account. The identification of the strain is made through the study of morphological and biochemical characters (urea-indole, (Tryptophan desaminase) (TDA), oxidase, catalase, (Orthonitrophenyl -β- galactosidase) (ONPG), mobility with (Brain heart broth) (BHB) and the reduced rack of Le Minor consisting of iron lysine, mannitol mobility, simmon citrate and the Hajna kligler).

The number of colonies per strains is evaluated in cfu (colony-forming unit) per (gram) of sample according to the following formula:

\[ N = \frac{\sum \text{colonies}}{V \text{(ml)} \times (n1+0.1n2) \times d1} \]

N: Number of sprouts per gram of initial product in CFU, refers to the sum of the colonies counted on all retained Petri boxe.
V: Volume of inoculum seeded to each Petri boxe in ml.
C: Number of colonies counted per Petri boxe on the two boxes selected.
n1: Number of Petri boxe read at the first dilution selected.
2.5 Macroscopic and Microscopic Identification of the Fungi Observed

Fungi are single or multicellular eukaryotic organisms lacking chlorophyll. They are distinguished by yeasts, molds and dermatophytes. In the environment, yeasts and molds are commonly found. In this study only molds were identified.

2.5.1 Macroscopic identification

The macroscopic study of the cultures was carried out by the observation, with the naked eye, of the cultural characteristics (aspect of the colony, color, recto, verso, and the speed of the growth). It is a question of observing the colonies of mold present on the Petri boxe containing Sabouraud chloramphenicol medium.

2.5.2 Microscopic identification

It has a blade and coverslip, put a drop of saline on the blade. A portion of the mildew colony is removed using a platinum loop in the presence of the flame and deposited on the slide, then spread until the colony is dissolved in the medium, covered with the coverslip and switch to identification with the electronic microscope. The reading is done by scanning at X10 magnification and then the details are fixed and specified at X40 magnification.

2.6 Stem Conservation

A portion of each fungal strain is removed from the Petri boxe or observed and then deposited in a screw tube containing distilled water. Storage at 4°C promotes viability and limits the possibilities of variations of fungal strains. It is recommended for short-term storage (6 months). For long-term conservation, the collected colony is placed in the Broth Heart brain Glycerol-10% and stored at -20°C at the Biobank.

2.7 Statistical Analyzes

For microbiological studies, the different results obtained are the average of three repetitions and are expressed as mean ± standard deviations. The data were subjected to one-way analysis of variance (ANOVA) using Graph Pad Prism 7 software (San Diego California, USA). The Dunnet test with a statistical significance threshold of p <0.05 was used for the comparison of means when the analysis of variance reveals significant differences.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of Infant Flours Studied

The results of the physicochemical characteristics of the various flours are shown in Table 2. The moisture content of the reference flour was 3.59 ± 0.2, and that of the local artisan flour was 6.56 ± 0.21. The moisture content of the various brands of infant flours of "Anagobaka" ranged from 8.79% ± 0.06 to 12.94% ± 0.08. The moisture content of the reference flour was significantly lower than that of the local and all the flours of "Anagobaka". However, the moisture content of local flour and those of "Anagobaka" flours do not meet the recommended standard for infant flour of 5%. The pH of the reference flour was 6.76 ± 0, 01 and that of the local flour 5.25 ± 0.01. The pH of the "Anagobaka" flours ranged from 5.94 ± 0.01 to 6.83 ± 0.01. All of the infant flour samples analyzed had acidic pH (Table 2). In addition, the pH values of most of these flours meet the recommended standard (5.0 - 6.8). Exempt those with the highest values.

3.2 Characteristics of the Allergenic Potential of Weaning Flours

3.2.1 Seeding in the mass

The microbiological results of the various flours samples after mass sowing and incubation indicate an absence of the studied germs for the reference flour, the artisanal flour and four infantile flours of "Anagobaka" Mesophilic aerobic germs were identified in seven different "Anagobaka" flours. Those are the brands flours Lady B, Jone Family, Bolero Strawberry, Fincap, Egg Banana, Tollex and Glad Family, whose load values were respectively 13.10^3 CFU / g ± 100; 3.37.10^3 cfu / g ± 17.32; 1.82.10^2 cfu / g ± 20; 1.28.10^2 cfu / g ± 20; 1.2.10^3 cfu / g ± 5.77; 1.19.10^3 cfu / g ± 10 and 5.10^2 cfu / g ± 100 (Table 3). There is a significant difference (P <0.05) between the loads of mesophilic aerobic organisms observed in these weaning flour samples.

3.2.2 Surface seeding

The results obtained after the surface seeding and the incubation of these different samples of
flours indicate an absence of the studied germs for the reference flour, the artisanal flour and five different brands of "Anagobaka" flours were identified in the same "Anagobaka" flours containing the mesophilic aerobic germs. The values of the charges were respectively $14 \times 10^2$ cfu / g ± 173.21; $11 \times 10^2$ cfu / g ± 200; $9 \times 10^2$ cfu / g ± 200; $6 \times 10^2$ cfu / g ± 100; $4 \times 10^2$ cfu / g ± 100 and $2 \times 10^2$ cfu / g ± 00 for the flours of Jone Family, Bolero Strawberry, Lady B, Finca, Egg Banana and Glad Family (Table 3). There is a significant difference ($P < 0.05$) between the fungal loads observed in these weaning flour samples.

### 3.3 Macroscopic and Microscopic Identification

Identification based on the macroscopic and microscopic analysis of fungal isolates on Sabouraud chloramphenicol medium of the various infantile flours samples made it possible to highlight the species *Aspergillus flavus* in all contaminated flours, one of which contained the *Aspergillus flavus* combination and *Mucor sp* (Table 4). Otherwise, the species *Aspergillus flavus* had a maximum contribution (54.54%) and appeared to be the most predominant species in "Anagobaka". The proportion of *Mucor sp* in flours was 9.10%.

**Macroscopic characters:** The *Aspergillus flavus* strains of flour A showed a white after yellow then yellow-green color with fluffy colonies and rapid growth and the optimum temperature was 37°C. Strains of the genus *Mucor sp* from flour C had a color that varied from gray to brown. They had very fast growing, extensive colonies with a woolly texture. The optimum growth temperature was 25°C.

**Microscopic characters:** Strains of *Aspergillus flavus* found in flour B had the following characteristics; vegetative propagation; conidiophores long, hyaline, warty with asperitites; spherical vesicles of 25 to 45 μm. Phialides are inserted directly into the vesicle, conidia are globose to subglobose, pale green, echinulate. The aspergillar heads are uniseriate and biseriate. The *Mucor sp* strains found in the D flour had broad, slightly septate filaments.

This part of our study aimed to evaluate the microbiological quality of the industrial flour used as a reference, the artisanal flour and the flour of different brands but well before, some physicochemical parameters had been determined.

Moisture is a very important parameter in taking into account the quality of an infant meal. It significantly affects the shelf life and growth of microbial contaminants in flour [18]. In fact, the low moisture content of flours limits the development of microorganisms, with the exception of molds [19]. In our study, the different flours had a high moisture content with no Mesophilic Aerobic Germs, Total Coliforms, Thermotolerant Coliforms, Staphylococci, Enterobacteria, *E. coli*, Sulphito-reducing Anaerobes and Salmonella. This could be explained by the roasting process. It is a technique that can inactivate antityrpsic factors, bacteria, reduce moisture, pre-bake products and extend the shelf life by 6 months [20]. Corinne (2013) [21] reported that good roasting takes place at around 150°C in about 20 to 30 min. Also a very long as a poor conservation of these infantile flours could generate a risk of contamination by germs of alteration.

The pH variation of a food favor or inhibit the growth of microorganisms in this food [22]. The acidic medium is responsible for inhibiting the growth of pathogenic microorganisms [23]. This explains the absence of bacterial microorganisms in our samples whose pH values were slightly acidic. The pH of the medium is an important factor in mold growth and mycotoxin production. Most molds grow in acidic pH and can tolerate very low pH values [24]. As confirmed by the presence of two types of mold, *Aspergillus flavus* and *Mucor sp* in more than half of the boxes of flours.

Concerning yeasts, they are usually present as contaminants in cereals and can develop at a very acidic pH where bacteria can not compete [25]. It is assumed that because of the low acidity of our samples, there was no yeast contamination. The absence of bacterial microorganisms and yeasts was observed in all the boxes of flours after microbiological analysis. However, two fungal genera (*Aspergillus sp* and *Mucor sp*) were identified during the study in six flours purchased in the markets of the District of Abidjan. The species *Aspergillus flavus* was present in all these samples and the genus *Mucor sp* was associated with the species *Aspergillus flavus* in one of the samples. These flours were made from corn cereals and came from Nigeria. Mycological investigations in Nigeria by Adebajo et al. [26] showed high levels of contamination in corn cakes. The dominance of the species
### Table 3. Microbial and fungal loads of different brands of infant weaning flours

<table>
<thead>
<tr>
<th>Flours</th>
<th>Gam</th>
<th>Entero</th>
<th>CT</th>
<th>CTH</th>
<th>E. coli</th>
<th>Staph</th>
<th>ASR</th>
<th>Y</th>
<th>M</th>
<th>Salmo</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolero P</td>
<td>&lt;10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Fincap</td>
<td>1.28.10^3±20</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(UMQ)</td>
</tr>
<tr>
<td>Jone Family</td>
<td>3.37.10^3±17,32</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(UMQ)</td>
</tr>
<tr>
<td>Tollex</td>
<td>1.19.10^3±10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Bolero strawb</td>
<td>1.82.10^3±20</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(UMQ)</td>
</tr>
<tr>
<td>Egg Banana</td>
<td>1.2.10^2±5,77</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(UMQ)</td>
</tr>
<tr>
<td>Queen Royale</td>
<td>&lt;10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Prime</td>
<td>&lt;10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Family Milk</td>
<td>&lt;10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Lady B</td>
<td>1.3.10^4±100</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(UMQ)</td>
</tr>
<tr>
<td>Glad Family</td>
<td>5.10^2±100</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(UMQ)</td>
</tr>
<tr>
<td>Farine de maïs</td>
<td>&lt;10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Cerelac Riz</td>
<td>&lt;10</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td>*</td>
<td>(VSMQ)</td>
</tr>
<tr>
<td>Norms FAO/OMS</td>
<td>&lt;10^4</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>Absence</td>
<td></td>
</tr>
</tbody>
</table>

The data are represented as mean ± SEM (n = 3). a, b, c, d; there is no significant difference between values designated by the same letter. GAM: Mesophilic Aerobic Germs; Entero: Enterobacteria; CT: Total Coliforms; CTH: Thermotolerant coliforms; E. coli: Escherichia coli; Staph: Staphylococci; ASR: Anaerobic Sulfito Reducers; Y: Yeasts; M: Molds; Salmo: Salmonella; *: 0 cfu / g; VSMQ: Very Satisfactory Microbial Quality; UMQ: Unsatisfactory Microbial Quality.
Table 4. Macroscopic and microscopic identification of fungal strains isolated from "Anagobaka" flours on Sabouraud chloramphenicol (Objective x 40) medium (Aspergillus flavus strains: flours A and B and Mucor sp strain: flours C and D)

Macroscopic appearance | Microscopic appearance
--- | ---
![A. flavus](image) | ![Microscopic A. flavus](image)
![Mucor sp](image) | ![Microscopic Mucor sp](image)

*Aspergillus flavus* is most commonly found on maize and cotton [27,28]. According to Lugauskas et al. [29], the frequency of contamination of corn flour by the genera *Aspergillus sp and Penicillium sp* was greater than 50%. Contamination by the genus *Aspergillus sp* was accompanied by mycotoxin production. Mycotoxins are toxic, low molecular weight secondary metabolites excreted by certain molds that develop on various products and habitat under particular conditions [30]. They diffuse into the substrate that they contaminate even after the destruction of the fungus responsible for their production. They are suspected in the occurrence of pathologies such as nutritional deficiencies, liver cancer, mutagenic, immunosuppressive and teratogenic effects [31]. Mycotoxins can cause diseases in animals and humans, they are involved in food poisoning [32]. They also alter the marketability of contaminated products. FAO estimates that 25% of agricultural products worldwide are contaminated with mycotoxins, causing significant economic losses [33]. We were unable to evaluate the concentration of mycotoxins in the contaminated samples in this study for technical reasons. As a result, the presence of mycotoxins in infant flours requires analytical techniques such as HPLC for detection limits of the order of 0.01 μg / kg.

During our work, the average percentage of mold contamination of *Aspergillus flavus* was 54.54%. The most common and most destructive fungi are *Aspergillus sp, Penicillium sp, Eurotium sp* and *Fusarium sp*. The genera *Aspergillus sp, Penicillium sp* and *Fusarium sp* are mycotoxin producers (aflatoxins, ochratoxins and
fumonisins), which are low-lability and often active secondary metabolites at very low doses [34,31]. Molds cause immunological and fungal infections in humans, particularly in vulnerable individuals such as young children, elderly people and the immunocompromised. The species *Aspergillus flavus* can cause invasive aspergillosis, aggravation of pathologies such as rhinitis or asthma [35,36]. It is known for its production of type B aflatoxins, the most frequent and toxic of which is aflatoxin B1 (hepatotoxic, teratogenic and mutagenic) [37]. Aflatoxins are thermostable mycotoxins that are resistant to sterilization processes. Amusa *et al* [38] showed that the presence of *Aspergillus flavus* in Ogi flours from Nigeria would produce aflatoxins involved in cancer of humans and animals. Dedi *et al*. [39] showed in their work that the various maize flours found on the markets of the Abidjan communes contained the molds of the genera *Aspergillus* sp, *Fusarium* sp, *Botrytis* sp, *Penicillium* sp and *Mucor* sp. *Mucor* sp genera quickly invade isolation media and hinder the growth of other fungal species. They colonize cereals, fruits and vegetables. The frequency of meal contamination by the genus *Mucor* sp was very low in our study and associated with *Aspergillus flavus* in the single box brand *Jone family*. Obi and Igbokwe [40] in their study isolated on the eggs several fungal strains which included the genus *Mucor* sp. The human diseases related to Mucorales are mainly infections of the ENT (sinusitis-rhinocerebral), pulmonary, cutaneous, subcutaneous and gastrointestinal infections. They are also at the origin of disseminated zygomycoses [41]. In developing countries several hypotheses may explain the contamination of infant flours by molds and their mycotoxins produced.

The presence of such a fungal storage flora suggests that contamination has probably occurred during the storage of cereal grains [42, 43]. Contamination of food by mold can occur along the entire chain: production, storage, processing, packaging and transport [44,45]. Some biotic (insects, mites and rodents) and abiotic (temperature, moisture) factors may influence fungal contamination of cereals [46]. In fact, all the molds found in these flour samples could come from the sales environment (proximity to roads, open-air sales, exposed products, etc.), the saleswomen themselves (dirty hands and clothes) or bad clothes storage conditions. Some studies have already indicated the presence of mold in flours sold in the markets of Abidjan district [39].

4. RECOMMENDATIONS

It is advisable to practice conservation techniques that are essentially based on the control of the physico-chemical or biological characteristics of the products or their environment. Examples include food additives. They are used to preserve the nutritional and sensory quality of the flours while extending the shelf life. Conservatives are used to protect foods against bacteria, yeasts or molds [47]. Unfortunately, some food additives whose synthetic dyes are toxic at high doses to the consumer. Resistant, stable and weatherproof packaging must be used to prevent any possible contamination. The polyethylene plastic packaging should be 60 to 80 μm thick for processed products with an expiration time of 6 months to 1 year [48]. The control of the nutritional, microbiological and toxicological quality of infant flours must be carried out before they are placed on our markets.

5. CONCLUSION

This study revealed that the microbiological and sanitary quality of the reference infant flour, the local infant flour and five infant flours are very satisfactory. However, the other six "Anagobaka" infant flours do not comply with the standard FAO / WHO, 2006 recommended of flour standards for moisture content and indicate the presence of molds of the species *Aspergillus flavus* and the genus *Mucor* sp which alter their microbiological quality. Within this fungal flora, *Aspergillus flavus* could produce aflatoxins and could be the cause of public health problems in infants and young children. Given the risks of exposure of the infant population to the mycotoxins produced by these molds, all stakeholders in the food production chain (farmers, producers, industrialists and consumers) should be sensitized. It would be imperative to improve the system of surveillance and control of local and imported infant flours. Finally, it will allow these infant flours to be real complementary foods to help cover the nutritional needs of young children.

ACKNOWLEDGEMENTS

Mrs Sylla K, engineer, Mycology lab; Mr Kissiedou, technician, food contaminants lab; Dr Diane K, epidemiologist, BioResource Center (BReCe); Pasteur Institute; Dr Zoro Bi Biochemistry Technical High School, Yopougon. Côte d'Ivoire.
CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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