



**Original article**

## Evaluation of the sanitary quality of peanut butters from Mali: Identification and quantification of Aflatoxins and pathogens

C. Keita<sup>a</sup>, A.H. Babana<sup>a,\*</sup>, D. Traoré<sup>a</sup>, F. Samaké<sup>a</sup>, A.H. Dicko<sup>a</sup>, F.A. Faradji<sup>a</sup>, K. Maïga<sup>a</sup>, A. Diallo<sup>b</sup>

<sup>a</sup>Laboratory of Research in Microbiology and Microbial Biotechnology, Faculty of Sciences and Technology, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali BP E3206.

<sup>b</sup>Agence Nationale de la Sécurité Sanitaire des Aliments (ANSSA), Bamako, Mali.

\*Corresponding author; Laboratory of Research in Microbiology and Microbial Biotechnology, Faculty of Sciences and Technology, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali BP E3206.

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### ABSTRACT

In Mali, peanut remains a major oilseed crops and constitutes in various forms a very important source of fat and protein for the Malian people. Despite the importance of peanut butters in the diet in Mali and the potential danger of its high consumption under certain conditions, little information exists in Mali on the quantities and qualities of aflatoxins, microbial contaminant and heavy metals present in peanut butters. The occurrence of aflatoxins, pathogens and heavy metals and microorganisms, mainly pathogenic bacteria, in 36 peanut butters from Mali was assessed and their microbial and toxicological quality determined. Aflatoxin B1 was found in 90% of the peanut butters tested at 2.34 to 189.34 µg/g of peanut butter). 50% of peanut samples analyzed exceeded the maximum allowable limit of aflatoxin B1. No sample was found to contain lead and Cadmium, the two heavy metals determined. Peanut butters from Kati were the contaminated with Salmonella and fecal coliforms, while no Salmonella or fecal coliform were detected in samples from Bamako. Fecal streptococci were not detected in ant peanut butter sample. Contrary, fungi and yeasts are present in all peanuts butter samples from Bamako and Kati. Significant positive correlation between grain types and the population of fungi were observed.

Contrary, a negative correlation was observed between grain types and yeasts. No lead or Cadmium was detected in the peanut butter samples from Mali.

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## 1. Introduction

The Food and Agricultural Organization of the United Nations (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (WHO, 1999). In recent years, incidences of food contamination by heavy metals and mycotoxins have become increasingly frequent raising question about their human health and economic consequences (Goyal et al., 2003; Kovacs, 2004; Wu, 2006).

In Mali, peanut remains a major oilseed crops. It constitutes in various forms (seeds, butter, oil, flour etc.) a very important source of fat and protein for the Malian people. Despite the importance of peanut butters in the diet in Mali and the potential danger of its high consumption under certain conditions, little information exists in Mali on the quantities and qualities of aflatoxins and heavy metals present in peanut butters. The presence of aflatoxins in peanut products in Africa has been recognized as a potential danger to human health (WHO, 2006). Among the naturally occurring aflatoxins (B1, B2, G1 and G2), aflatoxin B1 is the most important for humans and animals by its frequency and toxic compound, and is responsible for renal complications, immunological and cancérogénique. Ramjee et al. (1992) and Williams et al. (2004), pointed exposure to aflatoxin as a causal or aggravating factor for kwashiorkor in African children and suggested that chronic exposure to aflatoxins was associated with impaired immunity, malnutrition and liver cancer which is the third most common cause of death from cancer in Africa. Epidemiological studies of human populations exposed to diets naturally contaminated with aflatoxins revealed an association between the high incidence of liver cancer in Africa and elsewhere and dietary intake of aflatoxins (MERCK, 2006). A study on heavy metals level in Egyptian leafy vegetables showed that 97% of the leafy vegetables were contaminated with heavy metals with 39% exceeding the maximum limits for each element (Dogheim et al., 2004).

In addition, peanuts butters sold in Mali are generally prepared in a traditional way and under conditions of unhealthy hygiene. The conditions of production and storage of these peanut butters and especially their exposure to the air at the time of sale and packaging used are potential sources of contamination by pathogenic microorganisms. These contaminants can cause food poisoning and selling of poor-quality peanut butters in the markets. In Mali, peanuts butters are increasingly contaminated by pathogenic bacteria, fungi and yeasts (Babana et al., 2010). Also, the consumption of more and more of peanut butters expose more people to serious diseases (typhoid fever, diarrhea ....). So it is urgent to act, and quickly, to deal with such an eventuality.

## 2. Materials and methods

### 2.1. Selection and preparation of foods

Samples of peanut butter (2 kg) were purchased from four markets of Bamako (the capital of Mali) and from the main market and the factory in Kati city (Kayes region). The total number of samples tested was 198. The nature and number of each sample of food item, and the percentage of individual contaminant are presented in Tables 1 and 2, respectively. The samples were stored in sterilized bags, frozen and sent to the Central Laboratories at the Ministry of Public Health, in the State of Qatar for analysis. The samples were thoroughly mixed using a high powered Buchi mixer B-400 to give a large representative bulk.

For analysis, all the prepared samples were rapidly transferred to dry clean plastic containers with well-fitting closures, labeled and stored in freezers.

### 2.2. Analysis of samples

For aflatoxins identification, different samples were analyzed using the RIDASCREEN kit (R-Biofarm, Germany). Aflatoxins were extracted and identified according to the manufacturer's instructions (R-Biopharm, 2003). The different aflatoxins were extracted with methanol:water (70:30). The final extracts were diluted with distilled

water and used with the specific kit. The optical densities of the extracts were determined at 450 nm. All samples and standards were analyzed in duplicate. Data and mycotoxins in samples were analyzed using the software Ridasofwin of Biopharm.

The concentration of mycotoxins, in peanut butter samples and standards, were determined by the High Pressure Liquid Chromatograph (HPLC) equipped with a C18 column (5µm; 4.6 x150 mm), a fluorescent detector and a sampler. The quantification with HPLC was realized using as mobile phase the combination distilled water:acetonitrile:acetic acid (49.5:49.5:1) with a flow rate of 0.5ml by minute and  $\lambda_{exc}$  333 nm et  $\lambda_{em}$  450 nm. Aflatoxins concentrations were determined using calibration curve prepared for each identified aflatoxin (Frayssinet et Fremi, 1991).

Normal distribution of concentrations, means, standard errors and variances of identified aflatoxins were calculated using SAS software (SAS, 1990).

### **2.3. Determination of microbial population in peanut butters**

To determine the total coliform (TC), fecal coliforms (FC), the Salmonella (S) and Shigella we make the assumption tests and counting using the method of the Most Probable Number (MPN). The total coliforms were identified and counted after dilution (NF ISO 6887-1, 1999) following the method NF EN ISO 4831-1 (1991). Fecal coliforms were identified and counted according to the method NF EN ISO 4831-1 (1991). Fecal streptococci were identified and counted using a protocol close to that used for total coliforms determination, but the bacteria were grown on Azide Dextrose Broth medium. E. coli, has been researched and counted following the method NF ISO 7251 (1994). The Salmonella were identified and counted according to the method EN ISO / DIS 6579 (2001).

For yeasts and molds determination, ten (10) grams of peanut butter were scattered, using a mixer, in 90 ml of a solution of 0.05% Tween 80. 1 ml of each decimal dilution was plated on solid PDA medium and on solid PDA medium containing NaCl (PDA + 6% NaCl). Typical colonies were counted after 3, 5 and 7 days at 25°C and 31°C (Pitt, 1988; Rapper and Fennell, 1965; Botton, 1990).

### **2.4. Determination of the quality and level of aflatoxins in peanut butters**

To assess the occurrence of aflatoxins in peanut butter and determine their qualities and quantities, 50 g of each peanut butter sample was blended at high speed with 100 ml of 80% methanol/water for 3 minutes and filtered. The obtained filtrate was further diluted and processed as per procedure to obtain the final derivatized extract. It was injected into the HPLC column (NovaPack C18, 3.9 · 150 mm) at a flow rate of 0.5 ml/min using methanol: water:acetonitrile as the mobile phase and detected using the fluorescence detector (Ex.  $\lambda$  = 362 nm, Em.  $\lambda$  = 418 nm). The peaks obtained were then compared with sample peaks obtained with that of aflatoxin standards.

### **2.5. Determination of heavy metals in peanut butters**

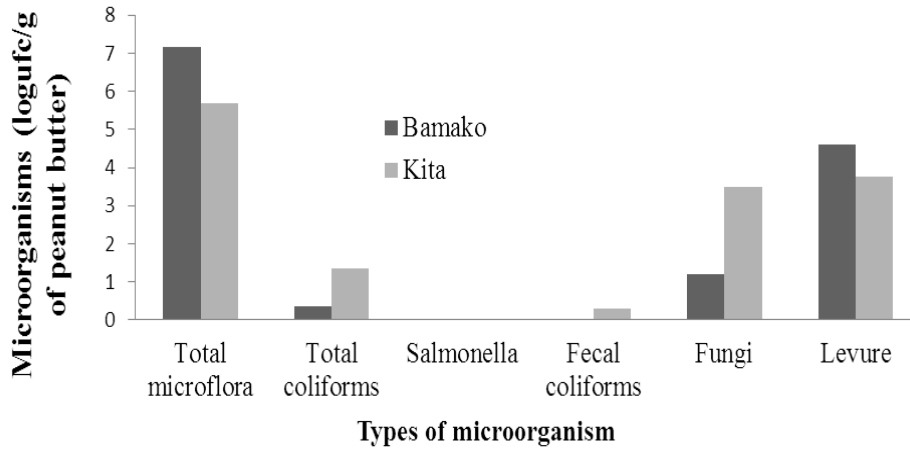
Heavy metals (lead and Cadmium) were analyzed using a Varian Spectra™ A880Z Zeeman AAS as stated in the AOAC (1990). In brief, about 500 mg of sample was microwave digested using 4 ml of concentrated HNO<sub>3</sub> and 1 ml of 30% H<sub>2</sub>O<sub>2</sub>. Digested sample was cooled and analyzed using the Zeeman AAS. For lead detection, lamp current was maintained at 5 mA, spectral band width at 0.5 nm and wavelength at 283.3 nm. For cadmium lamp current was maintained at 4 mA, spectral band width at 0.5 nm and wavelength at 228.8 nm.

## **3. Results**

### **3.1. Microbiological contamination of peanut butters**

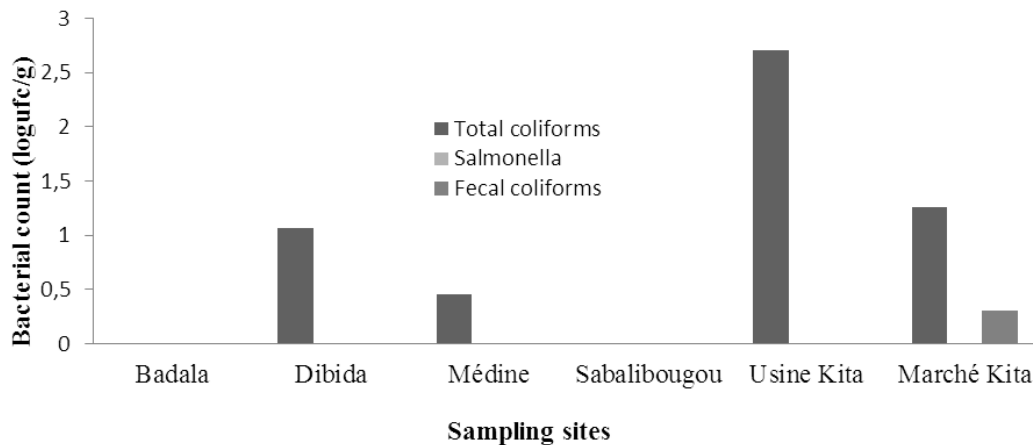
The contamination level of peanuts butter by microorganisms was determined according to standardized methods. Obtained results were presented on figures 1, 2 and 3.

Peanut butters from Bamako contain more total microorganisms than those from Kati (figure 1). But, peanut butter samples from Kati were most contaminated by pathogenic bacteria. In fact, since peanut butters from Kati were contaminated with Salmonella and fecal coliforms, no Salmonella or fecal coliform samples were detected in samples from Bamako. Contrary to pathogenic bacteria, fungi and yeasts are present in all peanuts butter samples from Bamako and Kati. But, fungi are more present on samples from Kati and yeasts were more detected on samples from Bamako (figure 1).



**Fig. 1.** Quantity and distribution of microorganisms in peanut butter from Bamako and Kati in Mali.

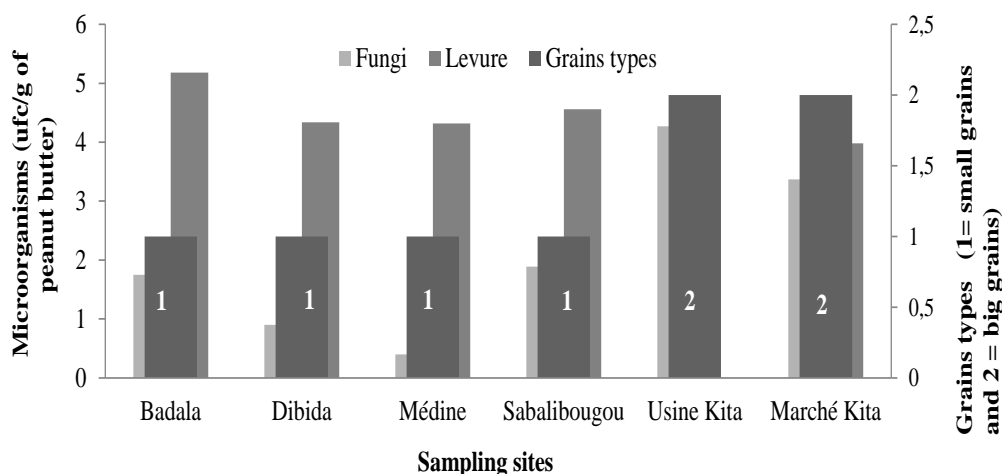
Contaminant and pathogenic bacteria in peanut butters from four market of Bamako and a market and factory from Kati in Mali were determined. Obtained results presented in figure 2, showed that peanut butter from the maket of Badala and the market of Sabalibougou were not contaminated and no pathogenic bacteria were detected in the peanut butters purchased from these two markets. The other peanut butters purchased in the markets of Dibida and Medine in Bamako and the market and the factory of Kita, were contaminated with total coliforms (figure 2). The peanut butter from the factory of Kita was the most contaminated with total coliforms. No Salmonella was detected in the peanut butters purchased in the markets of Bamako and in the market and factory of Kita. Fecal coliforms were detected only in the peanut butters purchased from the market of Kita. No fecal streptococcus was detected in any peanut butter samples analyzed.



**Fig. 2.** Population and distribution of contaminant and pathogenic bacteria in peanut butters purchased from different markets from Bamako and Kita and a factory from Kati.

Correlation studies carried out between the different microorganisms present in the analyzed peanut butter samples, showed significant positive correlation (0.91) between grain types (small or big grains) used to prepare peanut butters and the population of fungi. Contrary, a negative correlation (R=0.72) were observed between

grain types and levure content of peanut butter analyzed. These results showed also a negative correlation ( $R=0.74$ ) between Fungi and yeasts content of analyzed peanut butters.



**Fig. 3.** Distribution of Fungi and yeasts according to peanut grain types used to prepare peanut butter.

### 3.2. Quality and level of aflatoxins in peanut butter

90% of the peanut butters from Bamako and Kita were found to contain aflatoxin. The presence of aflatoxins in peanut products in Africa has been recognized as a potential danger to human health, even if it is caused by direct contamination from the seeds and seed products or accumulation of these toxins in animal tissues, meat and milk through the consumption of contaminated food. We conducted analyzes to identify and determine the amounts of aflatoxins present in each peanuts butter sample sold in Bamako and Kati. The results are shown in Tables 1 and 2.

**Table 1**

Qualité et taux d'aflatoxine dans les pâtes d'arachide vendues à Bamako et à Kita.

| Zones  | Aflatoxine B1 ( $\mu\text{g}/\text{kg}$ ) |
|--------|---|
| Bamako | $4 \pm 3,09$                              |
| Kita   | $34,08 \pm 26,42$                         |

**Table 2**

Qualité et taux d'aflatoxine dans les pâtes d'arachide vendues sur les différents marchés de Bamako et de Kita.

| Sites        | Aflatoxine B1 ( $\mu\text{g}/\text{kg}$ ) |
|--------------|---|
| Badala       | $5,15 \pm 3,72$                           |
| Dibida       | $4,08 \pm 2,26$                           |
| Médine       | $4,59 \pm 3,06$                           |
| Sabalibougou | $1,75 \pm 2,21$                           |
| Usine Kita   | $15,4 \pm 1,98$                           |
| Marché Kita  | $35,19 \pm 26,79$                         |

Analysis of the results reported in Table 1 showed that aflatoxin B1 was the main toxin in the peanut butters produced and sold in the markets of Bamako and Kita. An average of  $4 \mu\text{g}/\text{kg}$  and  $34.08 \mu\text{g}/\text{kg}$  of aflatoxin B1 were detected in peanuts butter, respectively in Bamako and Kita. Referring to the European standard which is  $5 \mu\text{g}/\text{kg}$  (Food Safety Authority in Ireland, 1999), we can say that on average, peanut butters purchased in Bamako are good

in terms of aflatoxin content. Instead, at the market and factory of Kita peanut butters were heavily contaminated by the aflatoxin B1. However, in both areas we observed great variations in the aflatoxin content of the peanut butter samples analyzed (Table 2).

Analysis of samples from: (i) four markets of Bamako and (ii) the market and the factory of Kita are shown in Table 3. These results showed that peanuts butter sold on the market of Kita are heavily contaminated with aflatoxin B1 (average  $35.19 \pm 26.79 \mu\text{g}/\text{kg}$ ). The strong variation in aflatoxin content indicates that taking samples from the same market one by one, some may contain up to  $62 \mu\text{g}/\text{kg}$  aflatoxin B1 while others do contain only about  $9 \mu\text{g}/\text{kg}$  aflatoxin B1. We found that despite the gap, all samples from the market of Kita contained more aflatoxin than the European standard. Similarly, samples of the factory of Kita contained more than the standard aflatoxin B1. Aside from peanut pasta sold in the market Badalabougou, pasta from other markets in Bamako contain normal doses of aflatoxin (Dibida  $4.08 \pm 2.26 \mu\text{g}/\text{kg}$  and Medina  $3.06 \pm 3.06 \mu\text{g}/\text{kg}$ ). Samples from the market Sabalibougou were the best with aflatoxin content ( $1.75 \pm 2.21 \mu\text{g}/\text{kg}$ ) very low compared to the standard. This study showed that special attention should be paid to peanut pasta sold in Mali. Since most of the Malian population (mainly Kita and Bamako) regularly consume peanut pasta, we can say that these people are at risk of poisoning with aflatoxin.

Correlations studies between different microorganisms present in the different peanut butter samples analyzed showed, on the one hand, a very strong positive correlation ( $r = 0.91$ ) between the types of grains used to produce peanut butters (small or mixed grains) and fungi content. On the contrary a negative correlation ( $r = 0.72$ ) was observed between the types of peanut grains used and yeast population in peanut butter. These results have shown a negative correlation ( $r = 0.74$ ) between yeast and fungi populations present in peanut butters sold in Mali (Figure 4).

### 3.3. Heavy metals

Lead and cadmium were not detected in peanut better sold in the markets of Bamako and Kita. That does not mean they are not present, but maybe they are at less quantities than the detectable limit for the analysis method used in this study.

## 4. Discussion

90% of peanut butter samples from Bamako and Kati contain detectable aflatoxin B1 in a range of  $0 \mu\text{g}/\text{kg}$  -  $189,29 \mu\text{g}/\text{kg}$ . The mean level of aflatonin B1 in peanut better analyzed was  $4 \mu\text{g}/\text{kg}$  in Bamako and  $34,086 \mu\text{g}/\text{kg}$  in Kita. This coincides with the understanding that the aflatoxin-producing fungus is particularly affiliated to peanut crops. The above results confirm those obtained by Adebajo (1993) who reported the presence of aflatoxin in tiger nuts (*Cyperus esculenta*) at toxicologically unsafe levels. Our results are, also, in the same line that those of Bassa et al. (2001) who detected aflatoxin in 98% of samples of dried yam chips surveyed in Benin with levels ranging from 2.2 to  $220 \mu\text{g}/\text{kg}$  and a mean value of  $141 \mu\text{g}/\text{kg}$ . These high contaminated peanuts butters made in home entered the local market distribution system, resulting in widespread aflatoxin contamination.

Peanut butter obtained from Kati was significantly contaminated by aflatoxin B1 levels greater than  $20 \mu\text{g}/\text{kg}$  compared to peanut butters bought from Bamako. Aflatoxin levels of about 30 times higher than  $10 \mu\text{g}/\text{kg}$  have been reported in peanut butter given to school children in Eastern Cape, South Africa (MERCK, 2006). A study by Gong et al. (2003) reported high contamination levels of staple foods by aflatoxins in West Africa. Akano and Atanda (1990) found aflatoxin B1 concentrations in the range of 20-455  $\mu\text{g}/\text{kg}$  in groundnut cake purchased from market in Ibadan, Oyo State, Nigeria. Similarly Adebajo and Idowu (1994) reported that most of the corn-groundnut snacks, contained aflatoxins above  $30 \mu\text{g}/\text{kg}$  immediately after preparation. Yamego and Kassamba (1999) reported that seeds of groundnut from Burkina faso inoculated with *Aspergillus flavus* excreted all the four major aflatoxins (B1, B2, G1 and G2) which peaked at  $170 \mu\text{g}/\text{kg}$  after six days.

Lead is a naturally occurring element that is hazardous when present at elevated concentration (Ona et al., 2006). Cadmium is a heavy metal, which is classified as a human carcinogen and is known to be toxic to plants (Deckert, 2005). In our study, lead and Cadmium contamination was not observed in the peanut butters purchased in Bamako and Kita. This is not in accordance to Ona et al. (2006) where plants were found to be capable of absorbing extra lead from soil.

The microbiological analysis showed that, peanut butters from Bamako contain more total microorganisms than those from Kati. But, peanut butter samples from Kati were most contaminated by pathogenic bacteria,

particularly fecal coliforms and Salmonella. This important contamination of peanut butter by pathogenic bacteria in Kati can be explained by the condition of preparation. These results can be explained by the contamination of peanut butters by the non-hygienic manipulation of these products. In fact, peanut butters were prepared by hand and in Kati water is not available in quantity to permit a proper hand washing by the producers and the vendors. These results can be explained by the contamination of peanut butters by the non-hygienic manipulation of these products. In fact, peanut butters were prepared by hand and in Kati water is not available in quantity to permit a proper hand washing by the producers and the vendors.

In this work, we also observed: (i) a very strong correlation between grain types and fungi population, (ii) negative correlations between grains types and yeast population and (iii) negative correlation between the populations of fungi yeast in peanut butters. In Bamako, all vendors claim to use the small grains (early varieties of peanut) to produce their peanut butter. These small seeds are known to contain significant amounts of oil which can prevent the growth of microorganisms. Instead, Kita vendors use a mixture of peanut grains (small + medium, medium + large or small + medium + large) to produce their peanut butter. Medium and large grains contain more dry matter and water and less oil than small seeds providing better growing conditions for microorganisms, mainly fungi.

## 5. Conclusion

The above analysis showed that the aflatoxin B1 is the main mycotoxin in peanut butter in Bamako and Kati in Mali. The potential hazard associated with aflatoxin B1 in peanut butter is more serious in Kati than in Bamako. The risks posed to health by the aflatoxin B1 in Kati can be lowered by reduced exposure by considering avoiding peanut and peanut products.

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