

Fungal Diversity of Food Supplements Sold on the Markets of Abidjan (Côte d'Ivoire): Case of Spirulina (*Arthrospira platensis*) and Moringa (*Moringa oleifera*) Powders

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To cite this article:

Yakoura Karidja Ouattara, Kalpy Julien Coulibaly, Sylvie Mireille Kouame-Sina, Zanakoungou Ibrahima Coulibaly, Amine Naty Tadiogo Kone, Vakou N'dri Sabine, Karidja Thanon, Eugene Patrice Kissiedou, Andre Offianan Toure, Adjehi Dadie. Fungal Diversity of Food Supplements Sold on the Markets of Abidjan (Côte d'Ivoire): Case of Spirulina (*Arthrospira platensis*) and Moringa (*Moringa oleifera*) Powders. *International Journal of Microbiology and Biotechnology*. Vol. 7, No. 1, 2022, pp. 43-50. doi: 10.11648/j.ijmb.20220701.16

Received: February 27, 2022; Accepted: March 21, 2022; Published: March 31, 2022

Abstract: The consumption of food supplements such as Spirulina and Moringa is increasing in developing countries. However, these foods, due to certain processing conditions, are likely to be contaminated by moulds potentially producing dangerous mycotoxins. The objective of this study is to determine the level of contamination and the diversity of fungal flora found in Spirulina (*Arthrospira platensis*) and Moringa (*Moringa oleifera*) powder produced and marketed in Abidjan. A total of 360 samples of powder, including 144 of Spirulina and 216 of Moringa, were collected from different sales outlets. The identification of fungal isolates was carried out on the basis of classical mycology criteria. The prevalence of fungal strains in the analyzed products was 85.83%, which 77.99% in Spirulina and 22.01% in Moringa. The predominant species were *Aspergillus flavus* (24.6%), *Aspergillus niger* (21.68%), *Penicillium sp* (20.71%), and *Aspergillus fumigatus* (12.62%). The study shows that Spirulina powder and Moringa powder sold in markets are contaminated with moulds, some of which are potentially mycotoxin producers posing a health risk to consumers. Strict hygiene measures must be observed during the production and marketing of Moringa and Spirulina powders in order to prevent any poisoning among consumers.

Keywords: *Moringa Oleifera*, *Spirulina Platensis*, Moulds, Mycotoxins

1. Introduction

Food supplements are defined as foodstuffs whose purpose is to supplement the normal diet. They are a concentrated source of nutrients or other substances with a nutritional or physiological effect, alone or in combination. These products

are marketed in the form of capsules, lozenges, tablets, powders and liquid ampoules [1]. Consumers, who are increasingly concerned about their health, are looking for these products as a way of compensating for (supposed or proven) deficiencies. This explains the expansion of the food supplement market [2].

Many plant resources have nutritional potentials that make them good candidates for food or nutritional supplements. This is the case of Moringa (*Moringa oleifera*) and Spirulina (*Arthrospira platensis*) used as food supplements [3]. Studies in different countries have proven the nutritional and therapeutic benefits of these two food supplements. Indeed, Moringa oleifera leaves contain a high concentration of vitamins, proteins, some minerals and possess the 10 amino acids and essential fatty acids [4]. In addition, the powder obtained from dried Moringa oleifera leaves has a higher concentration of nutrients than fresh leaves. It is therefore increasingly used as a food supplement by rural populations [5]. As for Spirulina (*Arthrospira platensis*), it is currently one of the best-known microalgae, studied and used as a food supplement in many countries [6]. It has a very remarkable composition [7]. It contains on average 70% protein, all essential amino acids, and high levels of iron, vitamins, and various elements necessary for the human body as well as antioxidant agents [8]. This microalga has received increased attention from researchers, mainly for its protein content, in addition to its use in the food, pharmaceutical, and cosmetic industries [9]. In addition, it has been touted as the "best food for the future" [6].

The benefits of Moringa and Spirulina are increasingly popularised through some media, websites, scientific conferences and word-of-mouth testimonies [10]. As a result, the marketing of these products is expanding in some health facilities, pharmacies, shops, supermarkets and markets. Moreover, the consumption of these food supplements is often perceived as being without risk. However, in some cases, it exposes the consumer to serious health risks due to the way they are produced and marketed. To this end, the 4 August 2017, ANSES issued an alert concerning the risks associated with the consumption of food supplements, particularly those containing spirulina. Moreover, the presence of certain fungal contaminants has been demonstrated in samples of Spirulina sold on markets [11, 12]. In addition, moulds have been observed in Moringa powder marketed for the benefit of People Living with HIV in Cotonou (Benin) [5]. Indeed, most of the dried foodstuffs are exposed to mould contamination during the drying, storage, handling and transport process until sale [13]. The main moulds involved are mycotoxin-producing fungi. They are ubiquitous in nature and possess a wide range of enzymatic arsenal, which allows them to grow on various substrates. Moulds reduce the technological (gluten content) and sanitary (allergy, toxic agents responsible for serious human and animal poisoning) quality of contaminated products. They also reduce the nutritional value by modifying the organoleptic aspect and cause economic problems due to the costs of detoxification or product rejection [14]. The identification of fungal species likely to colonise dehydrated foods and alter their qualities, through the production of mycotoxins, is an essential step in the evaluation of the mycotoxin risk. Thus, the study of the contamination of these foodstuffs by moulds potentially producing dangerous mycotoxins is very important. In this context and in view of the lack of scientific data on the mycoflora of Spirulina

(*Arthrospira platensis*) and Moringa (*Moringa oleifera*) produced and marketed in Côte d'Ivoire, it, therefore, seems interesting to carry out this study in order to determine the diversity of the fungal flora contaminating these two food supplements sold in certain markets in Abidjan.

2. Materials and Methods

2.1. Biological Material

The biological material consisted of Moringa powder (Figure 1) and Spirulina powder (Figure 2).



Figure 1. Moringa powder.



Figure 2. Spirulina powder.

2.2. Selection of Sampling Sites

As part of this study, an exploratory visit was made to markets in the thirteen communes of the Abidjan district, notably Abobo, Adjamé, Attécoubé, Anyama, Cocody, Koumassi, Marcory, Plateau, Port Bouët, Treichville, Songon, Plateau and Yopougon. This exploration was carried out to determine the availability and level of marketing of these food supplements. Subsequently, three communes including Adjamé, Yopougon and Cocody were selected for the purchase of Spirulina powder samples and six communes namely Abobo, Adjamé, Cocody, Koumassi, Marcory and Yopougon were selected for the purchase of Moringa powder

samples (Figure 3).

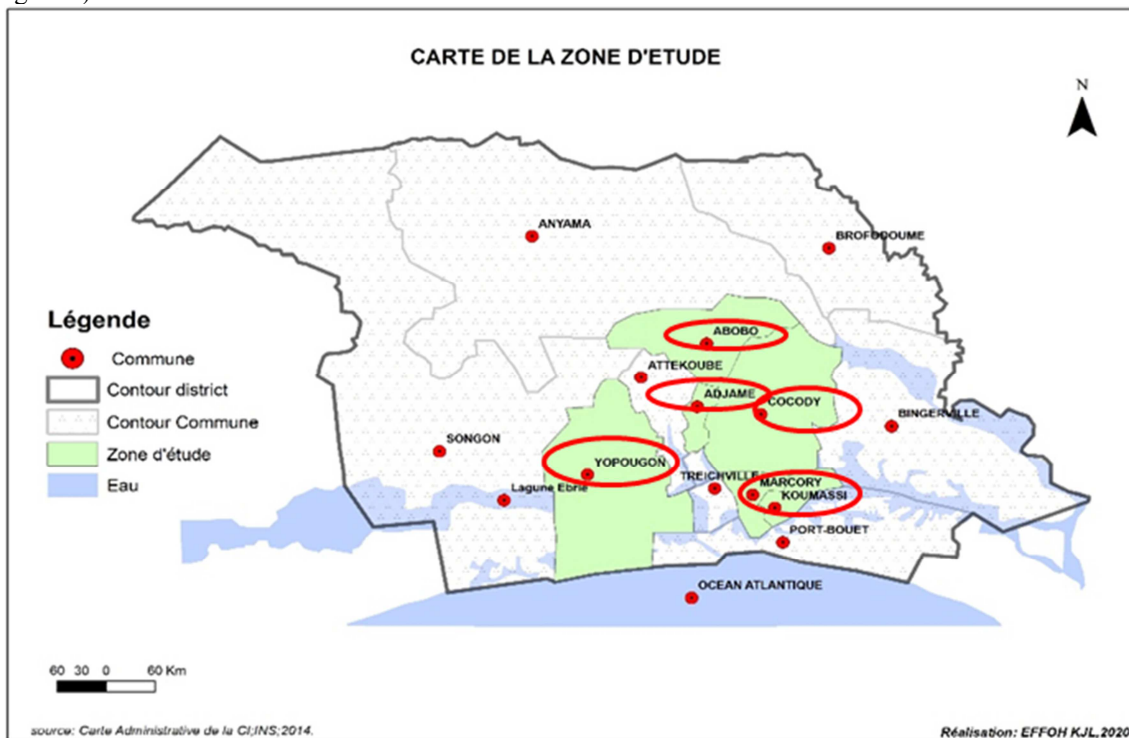


Figure 3. Sampling sites.

2.3. Sampling

Based on the availability of the product on the market, three vendors were selected at each site. A total of 360 samples were collected, including 144 *Spirulina* powder (48 samples per commune) and 216 *Moringa* powder (36 samples per commune) (Table 1). The samples were collected in their original packaging and aseptically at the different sales sites and sent to the laboratory of the Institut Pasteur de Côte d'Ivoire for mycological analysis.

Table 1. Distribution of samples collected by site.

		Vendor 1	Vendor 2	Vendor 3	Total
Abobo	Moringa	12	12	12	36
Adjame	Moringa	12	12	12	36
	Spirulina	16	16	16	48
Cocody	Moringa	12	12	12	36
	Spirulina	16	16	16	48
Koumassi	Moringa	12	12	12	36
Marcory	Moringa	12	12	12	36
Yopougon	Moringa	12	12	12	36
	Spirulina	16	16	16	48
Total	Moringa	72	72	72	216
	Spirulina	48	48	48	144

2.4. Isolation of Moulds

For the isolation of the moulds, a stock suspension was made by homogenising 10g of each sample in 90ml of Buffered Peptone Water (BPW). A series of decimal dilutions were made from this stock suspension and the dilutions 10⁻¹, 10⁻² and 10⁻³ were retained. Then 0.1 millilitre of these retained dilutions were spread on Sabouraud chloramphenicol

agar previously prepared and poured into Petri dishes. The plates were incubated at 25±2°C for 5 to 7 days [14]. Daily observations were made as soon as mycelium appeared. Each developed mycelium underwent several successive subcultures until pure strains were obtained, on each Petri dish of a single colony of a fungus [15]. The pure strains obtained were then subjected to mycological identification.

2.5. Identification of Isolated Moulds

All pure strains obtained were subjected to identification by classical mycological methods (macroscopic and microscopic identification). The macroscopic criteria were based on the observation of the colonies and their colour on both sides. For microscopic identification, the pure strain (a few spores and a mycelial fragment at the margin of the thallus) was taken with a sterile platinum loop and then placed on an object slide in a drop of physiological water. The whole was covered with a coverslip and observed under the objective 40 of the microscope, to define the microscopic criteria by using the specific determination keys of [16]. These criteria were based on the type of thallus (septate or not), the colour of the hyphae (dark or light), the shape of the spores, the origin of the spores (endogenous or exogenous), the shape of the heads (brush-like, aspergillate).

2.6. Statistical Analysis of the Data

The collected data were entered into the Excel spreadsheet and analysed using Statistica 7.1 software. A post-hoc comparison test with Anova LSD was performed when there were differences between the variables. Differences between

variables were considered significant at $p < 0.05$. The Excel spreadsheet was used to make the graphs. Past software was used to compare the diversity of mould populations identified in the samples collected, we calculated three diversity indices: Shannon's index (H') which measures diversity within a population and takes into account both richness and evenness, Simpson's index (D) with its opposite Simpson's diversity index ($1-D$), which gives more weight to common or dominant species, and the equitability index (E) which corresponds to the diversity of a stand where the taxa present would all have the same relative abundance.

3. Results

3.1. Level of Contamination of Spirulina Samples by Moulds

The analysis of the natural mycoflora of the different samples of Spirulina and Moringa powders revealed 309 strains of moulds, of which 241 strains were isolated from Moringa powders and 68 strains from Spirulina powders. The contamination rate of the Spirulina and Moringa powders analysed was 38.19% (55/144) and 86.57% (187/216) respectively. (Table 2).

Table 2. Level of mould contamination of Spirulina and Moringa samples.

		Samples analysed	Contaminated sample (%)	Number of fungal isolates
Abobo	Moringa	36	36 (100%)	51 (21,16%)
Adjamé	Moringa	36	33 (91,67%)	40 (16,60%)
	Spirulina	48	21 (43,75%)	27 (39,71%)
Cocody	Moringa	36	26 (72,22%)	31 (12,86%)
	Spirulina	48	13 (27,08%)	16 (23,53%)
Koumassi	Moringa	36	36 (100%)	50 (20,75%)
Marcory	Moringa	36	29 (80,56%)	33 (13,69%)
Yopougon	Moringa	36	27 (75%)	36 (14,94%)
	Spirulina	48	21 (43,75%)	25 (36,76%)
Total	Moringa	216	187 (86,57%)	241
	Spirulina	144	55 (38,19%)	68

3.2. Identified Mould Strains

Identification methods based on the macroscopic and microscopic characteristics of the 309 isolated strains identified the following fungal strains: *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus sp*, *Penicillium sp*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Fusarium sp* and *Scytalidium*

dimidiatum. The macroscopic and microscopic characteristics of the fungal isolates are recorded in Table 3. *Aspergillus flavus* was in the majority with a frequency of 24.6% (76/309) followed by *Aspergillus niger* with a frequency of 21.68% (67/309) and *Penicillium sp* with a frequency of 20.71% (64/309). (Table 4)

Table 3. Macroscopic and microscopic appearance of moulds contaminating Spirulina and Moringa.

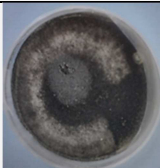
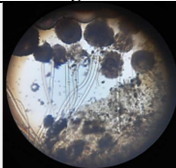
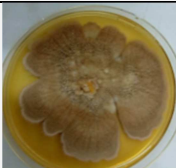
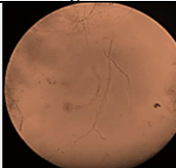
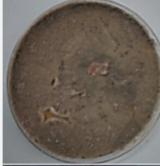

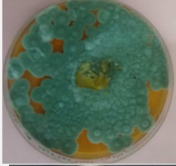
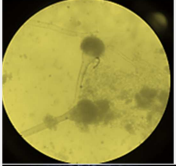

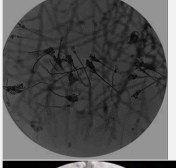

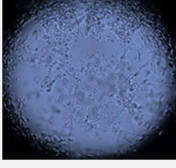
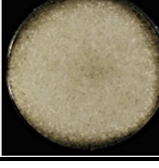
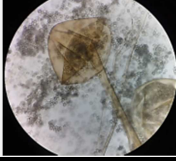
Species	Macroscopic aspect		Microscopic aspect at ×40 magnification	Macroscopic aspect		Microscopic aspect at ×40 magnification
<i>Aspergillus niger</i>				<i>Aspergillus terreus</i>		
<i>Aspergillus flavus</i>				<i>Aspergillus fumigatus</i>		
<i>Penicillium sp</i>				<i>Fusarium sp</i>		
<i>Rhizopus sp</i>						

Table 4. Frequencies of fungal species isolated from *Spirulina* powder and *Moringa* powder.

Species	Matrix	Number	Frequency of isolation	Effectifs
<i>Aspergillus flavus</i>	Moringa	42	17,43%	76 (24,6%)
	Spirulina	34	50%	
<i>Aspergillus niger</i>	Moringa	52	21,58%	67 (21,68%)
	Spirulina	15	22,06%	
<i>Aspergillus fumigatus</i>	Moringa	39	16,18%	39 (12,62%)
<i>Aspergillus terreus</i>	Moringa	18	7,47%	18 (5,83%)
<i>Penicillium</i> sp	Moringa	51	21,16%	64 (20,71%)
	Spirulina	13	19,12%	
<i>Fusarium</i> sp	Moringa	16	6,64%	16 (5,18%)
<i>Scytalidium dimidiatum</i>	Moringa	5	2,07%	5 (1,62%)
<i>Rhizopus</i> sp	Moringa	18	7,47%	24 (7,77%)
	Spirulina	6	8,82%	
Total	Moringa	241	100%	309 (100%)
	Spiruline	68	100%	

3.3. Abundance and Diversity of Mould Strains Isolated from *Moringa*

On the 241 mould strains identified in the *Moringa* powder, 62.66% (151/241) belonged to the genus *Aspergillus*; 21.16% (51/241) belonged to the genus *Penicillium*; 6.64% (16/241), 7.47% (18/241) and 2.07% (5/241) belonged to the genus *Fusarium*, *Rhizopus* and *Scytalidium* respectively (Table 5). Statistical analysis showed no significant difference between fungal isolates and sampling sites ($p > 0.05$). In order to compare the diversity of mould populations identified in the *Moringa* powder collected in the six communes, we calculated three diversity indices: Shannon's index (H') which measures diversity within a population and takes into account both richness and

evenness, Simpson's index (D) with its opposite Simpson's diversity index (1-D), which gives more weight to common or dominant species, and the equitability index (E) which corresponds to the diversity of a stand where the taxa present would all have the same relative abundance. The total indices for the sites were 1.91; 0.84; and 0.92 respectively for the Shannon index, for the Simpson index and for the equitability. The Shannon indices for all the sampling sites were greater than 1, which shows a high diversity of species. Simpson's indices for all sample sites ranged from 0.68 to 0.84, indicating the presence of a maximum number of isolated species at all sites. The equitability values across all the sample sites ranged from 0.80 to 0.93 which tends to 1 showing that the mould species present in all the *Moringa* powder samples have identical abundances (Table 6).

Table 5. Abundance of mould strains isolated from *Moringa* by sample origin.

Genus	Species	Abobo	Adjamé	Cocody	Koumassi	Marcory	Yopougon	Total
<i>Aspergillus</i>	<i>Aspergillus flavus</i>	10	5	10	7	8	2	42 (17,43%)
	<i>Aspergillus niger</i>	14	8	3	10	5	12	52 (21,58%)
	<i>Aspergillus fumigatus</i>	11	6	0	13	3	6	39 (16,18%)
	<i>Aspergillus terreus</i>	3	4	4	6	0	1	18 (7,47%)
<i>Penicillium</i>	<i>Penicillium</i> sp	7	10	6	5	8	15	51 (21,16%)
<i>Fusarium</i>	<i>Fusarium</i> sp	6	7	0	3	0	0	16 (6,64%)
<i>Rhizopus</i>	<i>Rhizopus</i> sp	0	0	6	4	8	0	18 (7,47%)
<i>Scytalidium</i>	<i>Scytalidium dimidiatum</i>	0	0	2	2	1	0	5 (2,07%)
Total		51	40	31	50	33	36	241

Table 6. Diversity index of mould strains isolated from *Moringa*.

Species	Abobo	Adjamé	Cocody	Koumassi	Marcory	Yopougon	Total général
<i>Aspergillus</i> sp	4	4	3	4	3	4	4
<i>Fusarium</i> sp	1	1	0	1	0	0	1
<i>Penicillium</i> sp	1	1	1	1	1	1	1
<i>Rhizopus</i> sp	0	0	1	1	1	0	1
<i>Scytalidium dimidiatum</i>	0	0	1	1	1	0	1
Taxas	6	6	6	8	6	5	8
Simpson_1-D	0,8368	0,6836	0,8035	0,8188	0,7908	0,7916	0,8361
Shannon_H	1,932	1,29	1,696	1,748	1,668	1,64	1,907
Equitability_J	0,9289	0,8014	0,9467	0,9757	0,9308	0,9156	0,9169

3.4. Abundance and Diversity of Mould Strains Isolated from *Spirulina*

On the 68 mould strains identified in the *Spirulina*

powder, 72.06% (49/68) belonged to the genus *Aspergillus*; 19.12% (13/68) belonged to the genus *Penicillium* and 8.82% (6/68) *Rhizopus* (Table 7). Statistical analysis showed no significant difference between fungal isolates

and sampling sites ($p>0.05$). In order to compare the diversity of mould populations identified in the Moringa powder collected in the six communes, we calculated three diversity indices. The total indices for the sites were 1.21, 0.66 and 0.87 for the Shannon index, Simpson index and equitability respectively. The Shannon indices for all sample sites were greater than 1, indicating high species

diversity. Simpson's indices for all sample sites ranged from 0.64 to 0.70, indicating the presence of a maximum number of isolated species at all sites. The equitability values in all the sampling sites ranged from 0.85 to 0.93 which tends towards 1 showing that the mould species present in all the Spirulina powder samples have identical abundances (Table 8).

Table 7. Abundance of mould strains contaminating Spirulina according to sample origin.

Genus	Species	Adjamé	Cocody	Yopougon	Total
<i>Aspergillus</i>	<i>Aspergillus flavus</i>	14	7	13	34 (50%)
	<i>Aspergillus niger</i>	6	4	5	15 (22,06%)
<i>Penicillium</i>	<i>Penicillium sp</i>	5	2	6	13 (19,12%)
<i>Rhizopus</i>	<i>Rhizopus sp</i>	2	3	1	6 (8,82%)
Total		27	16	25	68

Table 8. Diversity index of mould strains isolated from Spirulina.

Species	Adjamé	Cocody	Yopougon	General total
<i>Aspergillus sp</i>	2	2	2	2
<i>Penicillium sp</i>	1	1	1	1
<i>Rhizopus sp</i>	1	1	1	1
Taxas	4	4	4	4
Simpson_1-D	0,642	0,6953	0,6304	0,657
Shannon_H	1,18	1,282	1,133	1,211
Equitability_J	0,8511	0,9248	0,8174	0,8732

4. Discussion

Isolation of fungal strains from the Spirulina and Moringa powder samples yielded 309 fungal strains belonging to the genus *Aspergillus*, *Penicillium*, *Fusarium*, *Scytalidium* and *Rhizopus*. The samples were predominantly contaminated with moulds of the genus *Aspergillus* (*Aspergillus flavus* 24.6% and *Aspergillus niger* 21.68%). The high frequency of *Aspergillus* in Moringa powder and Spirulina could be explained by the fact that this fungus is found in the air and in the soil through the spores [17]. Furthermore, the presence of this fungus in all the samples taken in the different communes of the Abidjan district could be due to the fact that these food supplements come from the same production area. This suggests that the contamination of these products probably occurred either by the spores that were initially present in the production area, or later during storage or handling of the samples during the sale in the markets. Indeed, in the manufacturing process of food supplements such as Moringa, the leaves of this plant are washed, dried, crushed, stored and packaged [18]. Fungal contamination could have occurred during these manipulations. Similar results were observed in the work carried out by [19] on the Evaluation of the sanitary quality of Moringa oleifera leaf powders marketed for the benefit of people living with HIV in Cotonou (Benin). According to these authors, the presence of *Escherichia coli*, *thermotolerant coliforms*, yeasts and moulds in the samples tested suggests hygiene flaws in the production chain of Moringa leaf powders. In addition, flours are commonly contaminated with fungi. In their work on maize flour, [20] observed a contamination frequency of more than 50% by moulds of the *Aspergillus* genus.

Among the moulds isolated during this study, *Aspergillus*,

Fusarium and *Penicillium* are known as potential mycotoxin-producing strains, the best known of which are aflatoxins, ochratoxin and fumonisin, which are secondary metabolites that are not very labile and often active at very low doses [21]. Indeed, commonly dried and processed foodstuffs are significant targets for these toxigenic moulds [22]. The toxins can diffuse in the substrates they contaminate even after the destruction of the fungus responsible for their production [23]. The presence of such microorganisms in products intended for consumption is detrimental to the health of the consumer. Indeed, according to [24], these food and feed contaminants are the cause of various problems such as nutritional deficiencies, immunosuppression, mutagenic and teratogenic effects. Fungal strains producing aflatoxins Type B1, B2, G1 and G2, could be the cause of liver cancer according to [25]. Studies conducted by [26] confirmed the presence of *Aspergillus Niger* and *Aspergillus flavus* strains in grilled and dried "Kilichi" meats, these studies revealed the presence of aflatoxins of type B1, B2, G1 and G2 in the analysed samples secreted by *Aspergillus Niger* and *Aspergillus flavus*. In addition, the presence of ochratoxinogenic fungi such as *Aspergillus niger* and *Penicillium* argues in favour of ochratoxin A contamination [27]. This has been demonstrated with stored millet flour samples [28]. The stability of mycotoxins allows them to enter the food chain while maintaining their toxic properties [29].

5. Conclusion

This study shows that Spirulina and Moringa powders sold in the markets of Abidjan contain fungal species (*Aspergillus niger*, *Aspergillus flavus*, *Fusarium sp*, and *Penicillium sp*) that are potentially mycotoxin producers. These toxins are

sometimes incriminated in human and animal pathology. Furthermore, analysis of the nature and frequency of isolation of fungi shows a clear predominance of moulds of the genus *Aspergillus*. The presence of moulds in Spirulina powder calls for better control of processing techniques and compliance with good hygiene and manufacturing practices in order to reduce contamination and preserve consumer health.

Funding Information

We confirm that there was no funding for this study. It was self-financed by the authors.

Acknowledgements

We thank the Head of the Department of Mycology/Parasitology of the Institut Pasteur of Cote d'Ivoire, for allowing us to conduct this study. We also thank the sellers of Moringa powder and Spirulina for their collaboration in this study.

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