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Fungal contamination of Gabou Hamni sold in the markets of Niamey, Niger

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ABSTRACT

Gabou Hamni is a traditional condiment commonly used in Niger. It is formulated from different roasted vegetative parts of onion and roasted sesame. This study was conducted to determine the density of fungi and yeasts in Gabou Hamni sold at the markets of Niamey and also to identify the species of fungi present. A total of 25 Gabou Hamni samples were analyzed, of which 23 of them were purchased and 2 of them were prepared in the laboratory. The results obtained reveal that all samples purchased were contaminated by fungi and 48% by yeasts. Among the two samples prepared, only one was contaminated by fungi, but none was contaminated by yeasts. The density of fungi and yeast ranged from 0.5×10^2 to 1.65×10^4 CFU/g. Eight percent of the samples had fungi and yeast densities that exceeded the standard set by the International Commission on Microbiological Specifications for Foods. Nine genus of fungi were isolated. The fungi species identified from these genera were *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus flavus*, *Aspergillus nidulens*, *Penicillium citrinum*, *Penicillium citreonigrum*, *Rizopus stolonifer*, *Rizopus nigricans*, *Cunninghamella elegans*, *Absidia corymbifera*, *Phoma glomerata*, *Epicoccum purpurascens*, *Aureobasidium pullulans* and *Neosartorya fischeri*. The presence of these microorganisms constitute health risk. Exposures to the open air on market shelves, the use of obsolete and rudimentary packaging are probably the major factors of contamination by yeasts and fungi.

INTRODUCTION

Niger is an excellent onion producing country. Annual production is estimated at 711,964 tons (MA, 2013). Although the country is the sub region's leading onion exporter (Eplucher l'oignon, 2010), crop losses remain very high. They are mainly due to the high seasonality of the offer and the use of inefficient traditional conservation stores (Rabiou et al., 2018a). These stores, called *rudu* in Haussa language, have a loss rate that can exceed 70% (Prodex, 2012). In addition, there is a total absence of modern processing industries in the onion sector.

However, there is local know-how of onion processing and helps to minimize post-harvest losses. Onion is processed into Gabou, a spice commonly used in Niger. Gabou is obtained by roasting the different vegetative parts of the onion. Six types of Gabou are obtained depending on the vegetative parts of the onion used (Rabiou et al., 2018b). Gabou Hamni is the condiment form of Gabou. It is obtained after mixing, pounding and sieving of different types of Gabou with roasted sesame. Its formulation is a know-how at the discretion of the producer (Rabiou et al., 2018c). Gabou Hamni is

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composed of 7.81% moisture, 11.46% ash, 10.12% crude protein, 4.39% crude fat and 66.21% carbohydrates (Rabiou et al., 2018b). This condiment is used to season sauces in rural and urban areas. However, one of the major problems identified during the marketing of Gabou Hamni is the lack of appropriate packaging (Rabiou et al., 2019c). Gabou Hamni is exposed to the open air at the point of sale. This exposes it to all types of contamination. In addition, it quickly absorbs moisture due to its low moisture content (Rabiou et al., 2019b). This can promote the development of microorganisms (Tabuc, 2007). The presence of these microorganisms can not only alter the organoleptic but also the nutritional quality of Gabou Hamni. In addition, there may be secretion and accumulation of mycotoxins (Hashem and Alamri, 2010). The aim of this study was to determine the density of fungi and yeasts and also to identify the species of fungi present in Gabou Hamni. The identification of fungal species likely to colonize Gabou Hamni and alter its nutritional and organoleptic qualities, or even produce mycotoxins, is an essential prerequisite for assessing the mycotoxic risk associated with the use of Gabou.

MATERIALS AND METHODS

Sample collection

Samples were collected during the cold dry season. A total of 25 Gabou Hamni samples were analyzed, of which two (2) prepared in the laboratory (PL) and twenty-three (23) purchased from 23 sellers. The sellers are distributed over three (3) markets of Niamey, namely nine (9) resellers for the Harobanda market (HM), nine (9) for the Katako market (KM) and five (5) for the Djemadje market (DM). Gabou Hamni samples were collected aseptically in accordance with ISO 707.

Sample preparation

Twenty-five grams (25 g) of Gabou Hamni samples were introduced into 225 mL of tryptone salt (1 g of tryptone + 8.5 g of NaCl in 1 L of distilled water). The whole was mechanically homogenized at constant speed for 15 min to give a 10^{-1} solution (0.1 g /g of solution) (Moreira et al., 2009; Koohy-Kamaly-Dehkordy, 2013).

Inoculation and enumeration of fungi and yeasts

The standard ISO 7218:2001 (2001) setting out the general rules for microbiological examinations has been applied throughout the microbiological analysis process. The sabouraud agar glucose supplemented with Chloramphenicol previously prepared according to the manufacturer's instructions (Prolabo), melted and cooled is poured into a petri dish. After solidification, 0.1 ml of the 10^{-1} solution was spread over the entire surface of the Petri dish. Then the petri dish was incubated at 25°C for 5 days. Fungi and yeast colonies that appeared after 5 days of incubation were counted according to the standard ISO 21527-2:2008 (2008). The density of fungi and yeasts was expressed in Colony Forming Unit per gram of Gabou Hamni (CFU/g).

Identification of fungi species by the conventional method

The fungal isolates were transferred to sterilized plates for purification and identification. The grown fungi were mounted on a slide, stained with lactophenol-cotton blue to detect fungal structures (Basu, 1980), covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (Ronhede et al., 2005; Rajankar et al., 2007). The texts (books) used for identification of fungi, depending on their taxonomic keys are as follows; Moubasher (1993), Larone (1995), Pitt and Hocking (1997), Guarro et al. (1999), Howard (2002), Watanabe (2002), Ulhan et al. (2006) and Pornsuriya et al. (2008).

RESULTS AND DISCUSSION

To our knowledge, this study on the fungal quality of Gabou Hamni is the first. It is therefore new results that cannot be discussed and compared to previous knowledge. Figure 1 showed the density of fungi expressed in colony-forming unit per gram (CFU/g) of the 25 Gabou Hamni samples, of which twenty-three (23) purchased and two (2) prepared in the laboratory. The results showed that all samples purchased were contaminated by the propagules of various fungi. Among the two samples prepared, only one was contaminated by fungi. However, the fungi density of this sample was very low compared to those of the samples collected. The density of fungi in these samples varied from 1.0×10^{2} to 1.17×10^{4} CFU/g. The yeast density of Gabou Hamni samples also expressed in CFU/g was showed in Figure 2. The density of yeasts varied from 0 to 1.22×10^{4} CFU/g. 48% of samples were contaminated by yeasts. No prepared samples were contaminated by yeasts.

In order to judge the quality of the collected samples, the fungi and yeasts density were compared with the reference values. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1998), the fungi and yeasts density in spices is considered satisfactory up to 2.0×10^{3} CFU/g, acceptable between 2.0×10^{3} and 1.0×10^{4} CFU/g and not acceptable

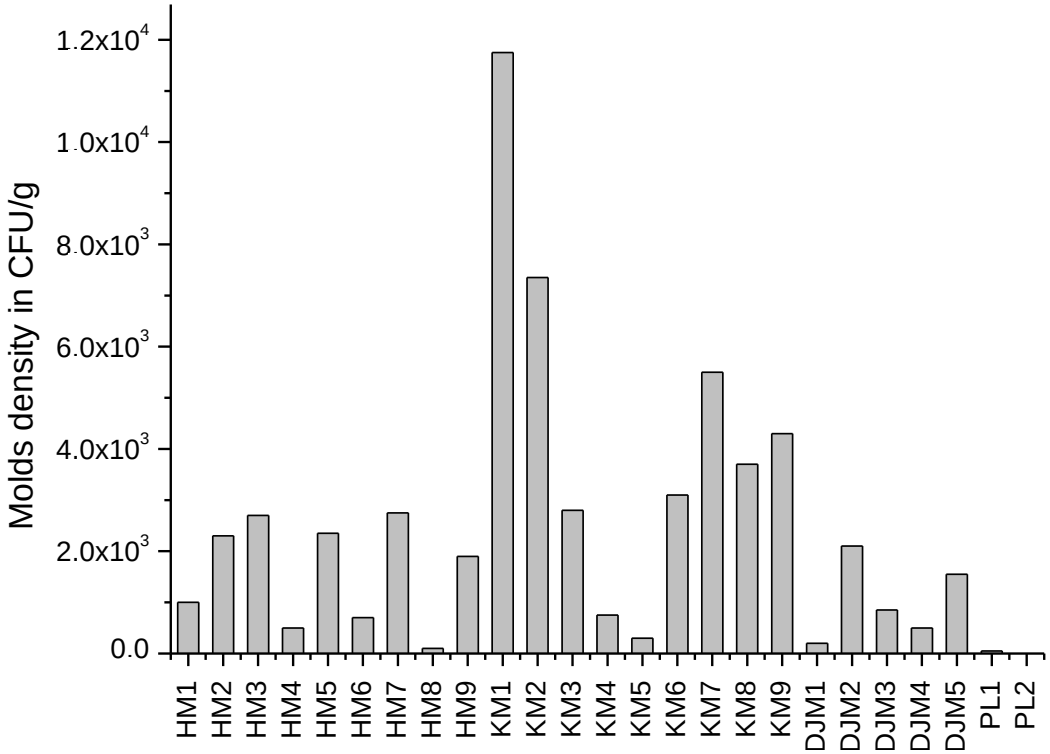

Figure 1. Fungi density in CFU/g of Gabou Hamni samples.

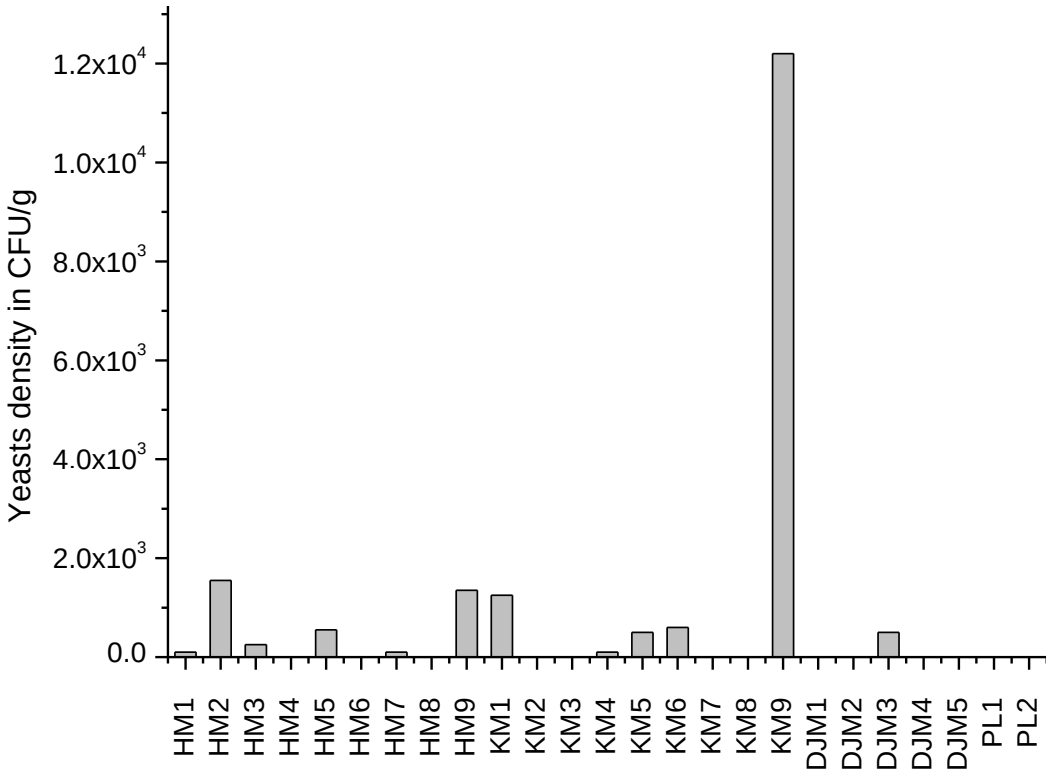

Figure 2. Yeasts density in CFU/g of Gabou Hamni samples.

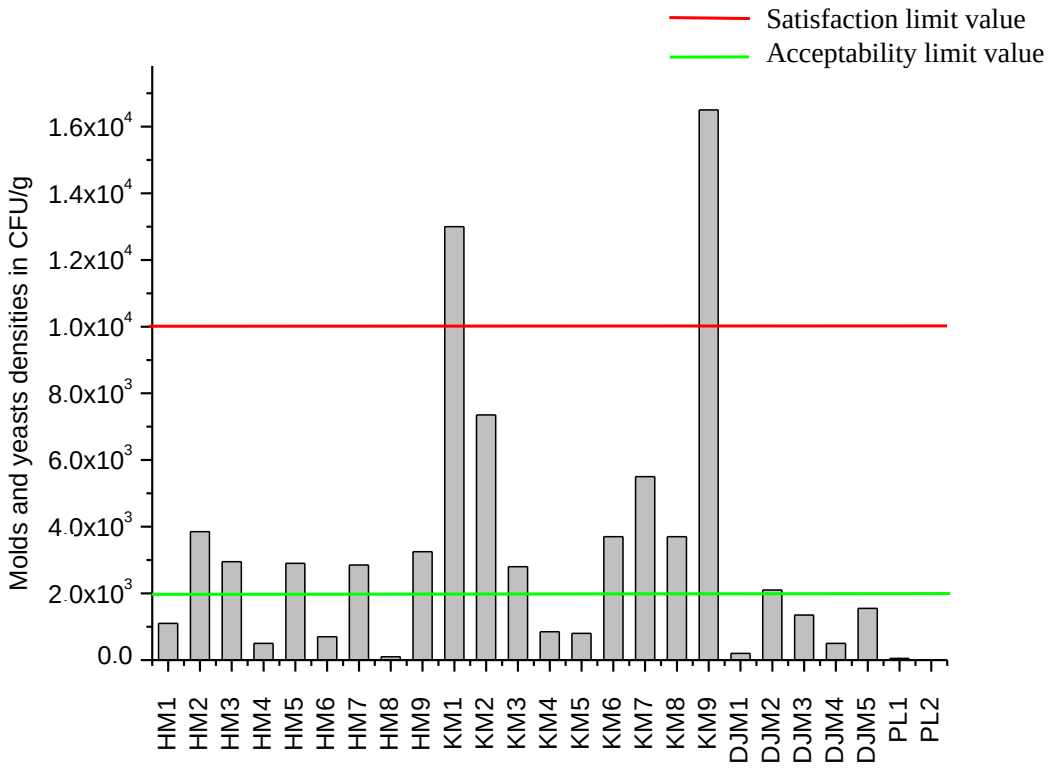

Figure 3. Fungi and yeasts densities in CFU/g of Gabou Hamni samples.

above 1.0×10^4 CFU/g. Figure 3 showed that 48% of the samples had a fungi and yeasts density of less than 2×10^3, so the quality of these samples could be considered satisfactory. 44% of the samples had a fungi and yeasts density between 2×10^3 and 1.0×10^4 and could be considered of acceptable quality. Whereas, 8% of the samples were out of the norm with a fungi and yeasts density superior to 1.0×10^4 CFU/g.

Gabou Hamni is a condiment with a low moisture content (Rabiou et al., 2018b) which makes the development of fungi less easy. However, the hazard may occur when added to the preparations because the conditions are in place to allow them to grow rapidly. The unwanted growth of fungi on a foodstuff is associated with multiple nuisances, including changes in the appearance of the food and its organoleptic and chemical characteristics (D'Mello and Macdonald, 1997). In addition, fungi can produce allergenic compounds and toxic metabolites (mycotoxins) that can affect consumer health (Nasser, 2001; AFSSA, 2009). Contamination of Gabou Hamni by fungi and yeasts can come from several sources. It may come from Gabou Hamni's exposure to air. Indeed, on the market shelves, this condiment is sold without packaging or in rudimentary and artisanal packaging. This exposes him to dust raised all day long. Many studies reported that exposure to open air, defective storage and the use of inadequate packaging are sources of contamination (Castegnaro and Pfohl-Leszkowicz, 2002; Larpent, 1996). High contamination of retail spices is considered an indication of environmental contamination (Koohy-Kamaly-Dehkordy, 2013). The low contamination obtained in some Gabou Hamni samples could be related to a short period of exposure to the open air. This is confirmed by the low fungi density of prepared Gabou Hamni samples, and bare-handed sampling of Gabou Hamni is another source of contamination (IGDA, 2001).

Figure 4 presented the number of contaminated Gabou Hamni samples by genus of fungi in percentage (%). This number varies from 8 to 72%. The results revealed also that Gabou Hamni's samples are contaminated by several genus of fungi. A total of nine (9) fungi genus, namely *Aspergillus, Rizopus, Penicillium, Absidia, Aureobasidium, Neosartorya, Epicoccum Cunninghamella* and *Phoma* were isolated from Gabou Hamni. The presence of these genus in *Gabo*u Hamni may represent a spoilage problem or a potential public health problem. In the latter, toxigenic fungi may produce toxins under certain conditions. In order to predict probable contamination by mycotoxins, species of genus isolated in the samples were identified. A total of fourteen (14) species were identified. Figure 5 showed that the number of contaminated Gabou Hamni samples by species varied from 4 to 56%.

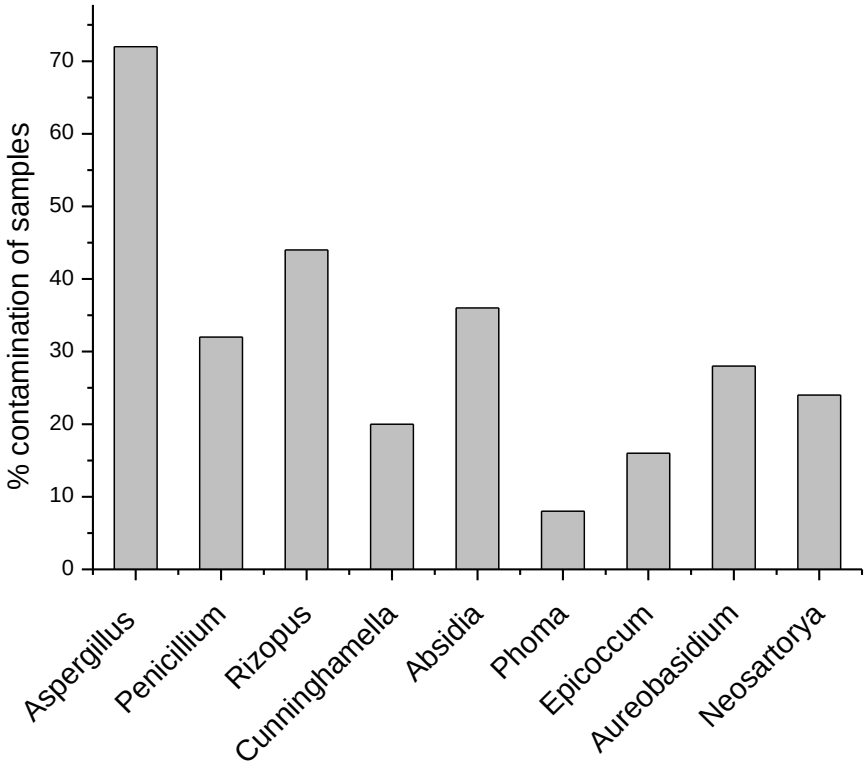

Figure 4. Frequency of contamination of Gabou Hamni by fungi genera.

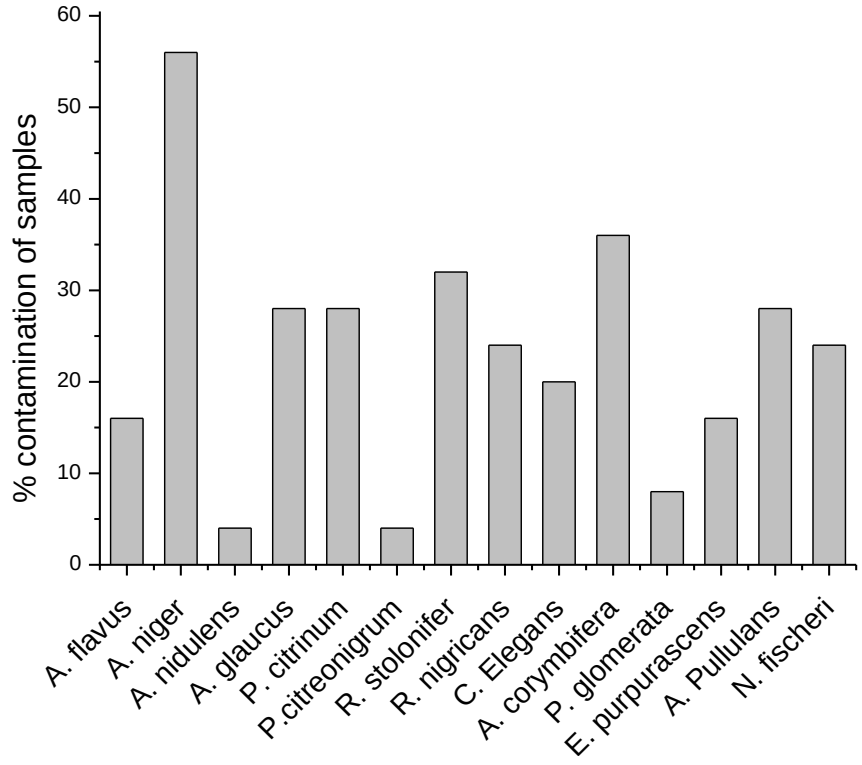

Figure 5. Frequency of contamination of Gabou Hamni by fungi species.

Among the isolated genus, *Aspergillus* was the most frequent in Gabou Hamni samples. It was found in 72% of samples. Our results were in well agreement with those of several authors who conducted studies on the microbiology of spices. According to these previous studies, *Aspergillus* was the predominant fungi isolated from most spices tested (Mandeel, 2005; Ruiz-Moyano et al., 2009). This genus was represented by four (4) species in Gabou Hamni samples. These species were *A. niger, A. glaucus, A. flavus and A. nidulens* (Figure 5). All these species are known aflatoxins producers (Belli et al., 2004; Pitt, 2000; Esteban et al., 2006a,b). Among these species isolated, *A. niger* was the most prevalent. This species was a very frequent fungal contaminant found worldwide on various substrates such as spices, cereals, but also grapefruits, or coffee bean (Toma and Abdulla, 2013; Battilani et al., 2006; Leong et al., 2007; Magnoli et al., 2007). *A. niger* was isolated in 56% of the samples at concentrations ranging from 1×10^2 to 18×10^2 CFU/g. *A. niger* was followed by *A. glaucus* which was isolated in 28% of the samples at concentrations of 1×10^2 to 6×10^2 CFU/g. 16% of samples contained *A. flavus*. This species count ranged from 1×10^2 to 7×10^2 CFU/g. *A. nidulens* was the less frequent in Gabou Hamni samples. Only 4% of samples were contaminated by this species. The total count of *A. nidulens* was 1×10^2 CFU/g. Data from literature showed that all these species were frequently isolated in spices (Toma and Abdulla, 2013; Ath-Har et al., 1988). *P. citrinum* and *P. citreonigrum* were the two species of the genus *Penicillium* isolated in Gabou Hamni samples. *P. citrinum* was presented in 28% of samples at concentrations 1×10^2 to 7×10^2 CFU/g whereas *P. citreonigrum* was presented in 4% at concentrations 1×10^2 to 2×10^2 CFU/g. These species produce mycotoxins (Tabuc, 2007). In this study, the *Rizopus* species identified were *R. stolonifer* and *R. nigricans. R. stolonifer* was detected in 32% of the studied samples at concentrations of 1×10^2 to 22×10^2 CFU/g whereas *R. nigricans* was contained in 24% at concentrations ranging from 1×10^2 to 6×10^2 CFU/g. The presence of this genus in the food testified a conservation under mediocre conditions (Larpent, 1996). *Absidia* was represented by *A. corymbifera*. This species was found in 36% of Gabou Hamni samples. *A. corymbifera* was the most encountered after *A. niger* in Gabou Hamni. The total count of *A. corymbifera* varied from 1×10^2 to 12×10^2 CFU/g. The predominant of *Aspergillus* (72%), *Rizopus* (44%), *Absidia* (36%) and *Penicillium* (32%) in Gabou Hamni samples was in accord with the results of Hashem and Alamri (2010), who reported that these fungal genus, *Absidia* excepted, were the most encountered in the spices analyzed.

Aureobasidium is an ubiquitous and cosmopolitan genus found. *A. pullulans* is the only species detected in studied samples. 28% of these samples were contaminated by this species. *A. pullulans* count varied from 2×10^2 to 5×10^2 CFU/g. In this study, 24% of Gabou Hamni samples were contaminated by *N. fischeri*. This species was the only found in the Gabou Hamni sample prepared in the laboratory. The presence of *N. fischeri* in the product prepared in the laboratory following strictly aseptic procedures testified to the resistance of this species to heat treatment. Previous studies reported that *N. fischeri* is heat-resistant species (Tournas and Traxler, 1994; Kotzekidou, 1997). *N. fischeri* count ranging from 2×10^2 to 22×10^2 CFU/g. *C. elegans* is a species of the genus *Cunninghamella*. This species was found in 20% of Gabou Hamni samples. The total count of *C. elegans* ranging from 1×10^2 to 6×10^2 CFU/g. Formerly known as *E. nigrum, E. purpurascens* was the only species of *Epicoccum* found in 16% of Gabou Hamni samples. The number of this specie varies from 1×10^2 to 4×10^2 CFU/g. *Phoma* is the fungal genus the less prevalent in the Gabou Hamni samples. It was found in 2 of 25 samples tested or 8%. *Phoma* was represented by *P. glomerata*, a specie that producers aflatoxins and acid kojic (Rai et al., 2009). The total count of *P. glomerata* was 2×10^2 CFU/g. *Aureobasidium, Neosartorya, Epicoccum Cunninghamella* and *Phoma* were the fungal genus less frequently encountered in Gabou Hamni samples. They were all represented by a single fungal species. Some of them are potentially mycotoxigenic (Nielsen et al., 1988; Rai et al., 2009). These species were isolated from various foods (Patterson et al., 2009; Samson et al., 2004). According to Kneifel and Berger (1994), fungi are the predominant contaminants of spices, but most such microbial populations are probably regarded as commensal residents on the plant that survived drying and storage. Soil and air is the main inoculums source for causing contamination in crude spices in field. Several authors reported that heating is sufficient for the elimination of fungi (Takano et al., 1985; Clear et al., 2002). Although Gabou Hamni was formulated from roasted ingredients, we note the presence of large numbers of fungi spores. This means that the combination of roasting temperature and time was not ideal for destroying fungus spores or else Gabou Hamni was contaminated during exposure to open air without packaging. The non-detection of fungi and yeasts, *N. fischeri* excepted, in the prepared samples, leads to the conclusion that Gabou Hamni collected from the markets was contaminated during exposure. Contamination is promoted by the use of bare hands during retail sale.

Conclusion

This study showed that many alteration species and potentially mycotoxin-producing species were present in Gabou Hamni samples collected from three markets in Niamey. The presence of these microorganisms in Gabou Hamni not only poses a health risk but also

reduces the market value of this condiment. There is an urgent need to raise awareness among Gabou retailers of the need to sell the product in sealed packaging but also to improve hygienic conditions for retail sales. The search for packaging that preserves hygienic, nutritional, technological and organoleptic qualities is necessary if not essential. It is also important to determine the mycotoxin content in Gabou Hamni. This will be the subject of a future study.

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