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REDUCTION OF AFLATOXINS AND MICROORGANISMS IN THE KOURA-KOURA PRODUCED IN BURKINA FASO WITH SPICES AND AROMATIC LEAVES

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ABSTRACT

Koura-koura is a product resulting from the processing of peanut. This study consisted of producing *koura-koura* with garlic, pepper, ginger and mint to reduce aflatoxins and microorganisms. The objective of this work is the decontamination of *koura-koura* with spices and aromatic leaves. Aflatoxin determination was performed by HPLC and microbiological analyses were carried out according to standard methods. A total of 18 samples were analyzed, including 3 peanut samples, 2 peanut paste samples, 1 *koura-koura* control sample, and 12 samples of *koura-koura* spices and aromatic leaves. Total aflatoxin B1, B2, and aflatoxin levels in the samples ranged from 0.58±0.49 µg/kg to 3.66±0.10 µg/kg; 2.23±0.41 µg/kg to 14.02±0.88 µg/kg; 2.87±0.20 µg/kg to 17.75±0.58 µg/kg, respectively. Aflatoxins G1 and G2 were not detected in all samples. The total mesophilic aerobic flora (TMAF) ranged from 1.60±1.57 $x10^1$ CFU/g to 4.50±1.28 $x10^5$ CFU/g and the yeast and mould flora ranged from 1.80 ± 1.68 $x10^1$ CFU/g to 2.80±0.74 $x10^1$ CFU/g. No samples were contaminated with thermo-tolerant coliforms and total coliforms were present in a single sample (1.30 ± 1.64 x 10^1 CFU/g). The results of the study on the reduction of aflatoxins and microorganisms in koura-koura with spices and aromatic leaves contribute significantly to food safety.

Contribution/Originality: This study on the reduction of aflatoxins and microorganisms in the *koura-koura* contributes to the production of healthy food. Our study uses aflatoxin control means that are effective, very easy and inexpensive.

1. INTRODUCTION

Peanuts are very sensitive to mycotoxins. These mycotoxins are made from microscopic fungi or moulds belonging mainly to the *Aspergillus* and *Penicillium* type which proliferate mainly in hot and humid regions such as in our tropics (Wacoo, Wendiro, Vuzi, & Hawumba, 2014). Human ingestion of these mycotoxins causes many health problems. Aflatoxins cause liver cancer (HCC) or "hepatocellular carcinoma" which is the most common cancer in the world and the third most common cause of cancer death worldwide (Bbosa et al., 2013).

Other studies have also highlighted the role of aflatoxins in the development of other types of cancer such as kidney, respiratory and gastrointestinal (GIT) cancers (Bbosa et al., 2013; Cui et al., 2015). Aflatoxin is found in processed products because it is resistant to various processing and preservation processes. Thus, conservation

processes (sterilization, pasteurization, freeze-drying, freezing), if they act on moulds, do not destroy mycotoxins or very little (Bullerman & Bianchini, 2007).

Preventive methods have shown their limitations because mycotoxins are always present in peanut seeds, the use of methods to reduce mycotoxins in *koura-koura* is imposed for better health of consumers. Thus, for post-harvest interventions, strategies focus on decontaminating substrates after toxin synthesis or reducing availability by limiting uptake by exposed organisms (El Khoury, 2016). Some physicochemical processes such as grinding, brewing, roasting, nixtamalization, extrusion, irradiation, ozonation, etc. have been tested to reduce mycotoxin levels in foods (Bullerman & Bianchini, 2007; Chen et al., 2014; Riley & Norred, 1999; Wang et al., 2016).

However, these methods are still insufficient to guarantee food safety. The use of natural compounds, generally recognized as safe for the environment and health, is a good alternative because plants produce various secondary metabolites (terpenoids, phenolic compounds, alkaloids, etc.) for their protection against attacks of any kind (mechanical, biological, or climatic) (El Khoury, 2016). The use of local spices and aromatic leaves (pepper, garlic, ginger, mint) for the detoxification of the peanut paste used to produce *koura-koura* has several advantages for the country because they are rich in antioxidants, available (all year round), inexpensive, safe for the health of the consumer, likely to improve the nutritional and organoleptic qualities. A *koura-koura* with improved nutritional and organoleptic qualities is essential to ensure food security.

2. MATERIALS AND METHODS

2.1. Sampling

Koura-koura samples used for analysis come from our productions following the experimental diagram. A quantity of 500 g was taken from each production for analysis. To avoid any contamination, they were put in stomacher bags and stored at 4° C.

2.2. Plant Material

Peanut is the main plant material that has been used for the production of *koura-koura*. The spices and aromatic leaves that were used in the production of *koura-koura* were: garlic, ginger, pepper, and mint. Peanut, spices and aromatic leaves were purchased in local markets in the city of Ouagadougou.

2.3. Development of the Production Diagram

A follow-up of the *koura-koura* production with the women producers made it possible to collect information on the different methods and stages of production. This information collection made it possible to draw up the production diagram.

2.4. Spices and Aromatic Leaves Used

Detoxification tests consisted of incorporating into the *koura-koura* the percentages 1%, 2%, and 3% (w/w) for each spice or aromatic leaf (Ayoade and Adegbite, 2016).

2.5. Effects of Different Unit Operations on Aflatoxins and Microorganisms

Samples were taken at each stage of production (raw peanuts, roasted peanuts with skins, roasted peanuts without skins, peanut paste, de-oiled peanut paste, and *koura-koura*). These different samples were assayed for aflatoxins and total mesophilic aerobic flora, yeasts and moulds, total coliforms, and thermos-tolerant.

2.6. Aflatoxin Dosage

Aflatoxin was determinated according to ISO-16050 (2003). Immunoaffinity columns were used for the purification of aflatoxin B1, B2, G1, and G2 samples before HPLC. The column used was ZOBAX-SB-C18 4.6x255

mm. The HPLC parameters were: mobile phase flow rate: 1.0 ml/min; injection volume: 50 µl; excitation / emission: 365/435 nm.

2.7. Microbiological Analyses

The preparation of *koura-koura* samples was monotored according to ISO 6887-6 (2013). Total mesophilic aerobic flora was counted on Plate Count Agar medium according to ISO 4833 (2003). Yeasts and moulds were counted on Sabouraud chloramphenicol agar medium (ISO 21527-2, 2008). Total and thermos-tolerant coliforms were enumerated on the Violet Red Bile Lactose Agar medium at 30°C and 44°C respectively for 24 h to 48 h (ISO 4832, 2006).

2.8. Statistical Analysis

Data from the microbiological analyses, aflatoxin essay, hierarchical ascending classification, and analysis of the main components were analyzed using XL STAT software. Statistical analysis was performed using the ANOVA test and the dendo test. The difference between the means is significant when $p < 0.05$.

3. RESULTS

3.1. Production Diagram of Koura-Koura

The *koura-koura* production diagram is shown in Figure 1. Different productions have been carried out according to the traditional production process whose unitary operations are: sorting/cleaning: it eliminates defective seeds, solid waste (stones, rotten peanuts, sand, straw,). Roasting: it is a thermal action that consists of lightly roasting the peanut seeds. Depelliculage: consists of removing the skin and germs from the cotyledons by rubbing. Crushing: consists of reducing the cotyledons to a paste using an electric grinder. Mixing/shaping: Mixing removes a large quantity of oil. Shaping consists of giving different shapes to the de-oiled paste. Frying: consists of cooking the shaped peanut paste in oil.

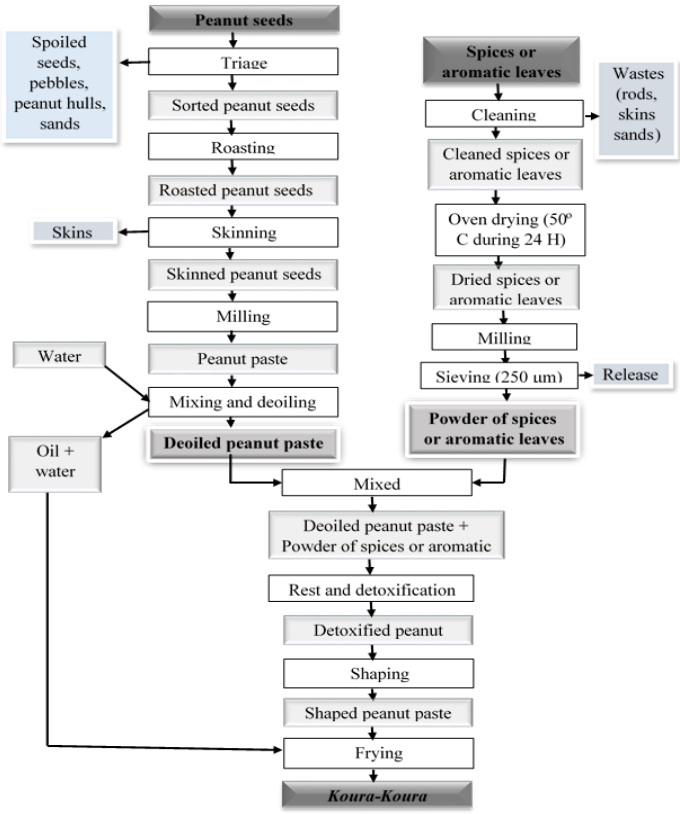

Figure-1. Production diagram of *koura-koura* .

3.2. Aflatoxin Contents in Samples

The aflatoxin levels of the different peanut and *koura-koura* samples are recorded in Table 1. These results show the effect of different unit operations on aflatoxin. The concentration of aflatoxin B1 ranged from 2.09 ± 0.62 µg/kg (KKT) to 3.72 ± 0.70 µg/kg (PA). The concentration of aflatoxin B2 ranged from 9.22 ± 0.50 µg/kg (KKT) to 14.02 ± 0.88 µg/kg (PA). Total aflatoxin content ranged from 11.31 ± 0.12 µg/kg (KKT) to 17.75 ± 0.58 µg/kg (PA). Aflatoxins G1 and G2 were not detected in all samples. The differences between the means are significant ($p < 0.05$).

Table-1. Aflatoxin contents of the different peanut and *koura-koura* samples.

Designation	Aflatoxin B1 (µg/kg)	Aflatoxin B2 (µg/kg)	Aflatoxin G1 (µg/kg)	Aflatoxin G2 (µg/kg)	Total Aflatoxin (µg/kg)
AC	ND	ND	ND	ND	ND
AT	ND	ND	ND	ND	ND
ATD	ND	ND	ND	ND	ND
PA	3.72 ± 0.70	14.02 ± 0.88	ND	ND	17.75 ± 0.58
PAD	3.29 ± 0.48	13.20 ± 0.12	ND	ND	16.49 ± 0.60
KKT	2.09 ± 0.62	9.22 ± 0.50	ND	ND	11.31 ± 0.12
Norm of European Commission: Maximum concentration of aflatoxins (B1+B2+G1+G2) of peanut: 15 µg/kg according to the product and the transformation proceeding					

Note: Legend: AC: Raw peanut; AT: Roasted peanut; ATD: Roasted and Skinned peanut; PA: Peanut paste; PAD: Paste Deoiled peanut paste; KKT: *koura-koura*; ND: Not detected.

3.3. Different Flora in the Samples

Counts of the different floras in the peanut and *koura-koura* samples yielded values that are recorded in Table 2. The total mesophilic aerobic flora (TMAF) ranged from 4.38 ± 1.92 x 10^2 CFU/g (KKT) to 4.50 ± 1.28 x 10^5 CFU/g (CA). Yeast and mould flora ranged from 1.80 ± 1.68 x 10^1 CFU/g (KKT) to 2.80 ± 0.74 x 10^1 CFU/g (CA). Thermo-tolerant coliforms were absent. Total coliforms were present in the AC sample (1.30 ± 1.64 x 10^1 CFU/g).

Table-2. Microbiological results of the different peanut and *koura-koura* samples.

Samples	TMAF (CFU/g)	Yeasts and moulds (CFU/g)	Total coliforms (CFU/g)	Thermo-tolerant coliforms (CFU/g)
AC	4.50 ± 1.28 x10^5	2.50 ±1.39 x10^1	1.30 ± 1.64 x10^1	ND
AT	4.65 ± 0.42 x10^3	1.90 ±1.96 x10^1	ND	ND
ATD	4.29 ± 1.56 x10^3	1.70 ± 0.43 x10^1	ND	ND
PA	4.19 ± 1.85 x10^5	2.80 ± 0.74 x10^1	ND	ND
PAD	4.10 ± 0.64 x10^5	2.10 ± 0.34 x10^1	ND	ND
KKT	4.38 ± 1.92 x10^2	1.80 ±1.68 x10^1	ND	ND
Threshold limit (2005/2073/CE)	10^6	10^4	10^3	10

Note: Legend: ND: Not detected.

3.4. Aflatoxin Content of Koura-koura with Spices and Aromatic Leaves

The aflatoxin concentrations of *koura-koura* produced with spices (garlic, pepper, ginger) and aromatic leaves (mint) are shown in Table 3. Aflatoxin B1 content of *koura-koura* samples ranged from 0.58 ± 0.60 µg/kg (KKM3) to 1.74 ± 0.84 µg/kg (KKG1); aflatoxin B2 content ranged from 2.23 ± 0.82 µg/kg (KKP3) to 7.64 ± 0.16 µg/kg (KKG1). The total aflatoxin concentration ranged from 2.87 ± 0.75 µg /kg (KKP3) to 9.38 ± 0.08 µg/kg (KKG1). Aflatoxins G1 and G2 were not present in the samples. The differences between the means are significant ($p < 0.05$).

Table-3. Aflatoxin content of *koura-koura* with spices and aromatic leaves.

Code	Aflatoxin B1 (µg/kg)	Aflatoxin B2 (µg/kg)	Aflatoxin G1 (µg/kg)	Aflatoxin G2 (µg/kg)	Total aflatoxin (µg/kg)	Percentage reduction of total aflatoxins (%)
KKA1	0.99 ± 0.98	3.92 ± 0.46	ND	ND	4.91 ± 0.44	56.58
KKA2	0.97 ± 0.30	3.45 ± 0.58	ND	ND	4.42 ± 0.88	60.91
KKA3	0.96 ± 0.88	3.25 ± 0.12	ND	ND	4.22 ± 0.02	62.68
KKP1	1.33 ± 0.56	5.41 ± 0.04	ND	ND	6.74 ± 0.60	40.40
KKP2	1.18 ± 0.96	4.76 ± 0.30	ND	ND	5.95 ± 0.26	47.39
KKP3	0.64 ± 0.68	2.23 ± 0.82	ND	ND	2.87 ± 0.75	74.62
KKG1	1.74 ± 0.84	7.64 ± 0.16	ND	ND	9.38 ± 0.08	17.06
KKG2	1.60 ± 0.32	6.89 ± 0.86	ND	ND	8.49 ± 0.18	24.93
KKG3	1.37 ± 0.58	5.68 ± 0.88	ND	ND	7.05 ± 0.38	37.66
KKM1	0.71 ± 0.22	2.56 ± 0.87	ND	ND	3.78 ± 0.30	68.43
KKM2	0.63 ± 0.02	2.42 ± 0.66	ND	ND	3.05 ± 0.68	73.03
KKM3	0.58 ± 0.60	2.34 ± 0.42	ND	ND	2.93 ± 0.02	74.09
Regulation of Food and Drug Administration (FDA): Maximum concentration of aflatoxins for food for human consumption: 20 µg/kg						

Note: Legend: KKA1: *koura-koura* with garlic 1%; KKA2: *koura-koura* with garlic 2%; KKA3: *koura-koura* with garlic 3%; KKP1: *koura-koura* with pepper 1%; KKP2: *koura-koura* with pepper 2%; KKP3: *koura-koura* with pepper 3%; KKG1: *koura-koura* with ginger 1%; KKG2: *koura-koura* with ginger 2%; KKG3: *koura-koura* with ginger 3%; KKM1: *koura-koura* with mint 1%; KKM2: *koura-koura* with mint 2%; KKM3: *koura-koura* with mint 3%; ND: Not detected.

The Principal component analyses reveal that the F1 and F2 axes report 99.93% of the information on the aflatoxins content in the analysed samples Figure 2. Thus, vectorially, aflatoxin B1 is opposed to total aflatoxin and aflatoxin B2. Aflatoxins G1 and G2 are not active variables in the construction of the axes F1 and F2. According to the axis F1, samples KKG2, KKG1, KKG3, KKP1 contained high quantity of aflatoxin B1 and B2. Unlike of samples KKP2, KKP3, KKA2, KKA3, KKM3, KKM1, KKM2, KKA1 which have low content of aflatoxins B1 and B2 according the axis F2.

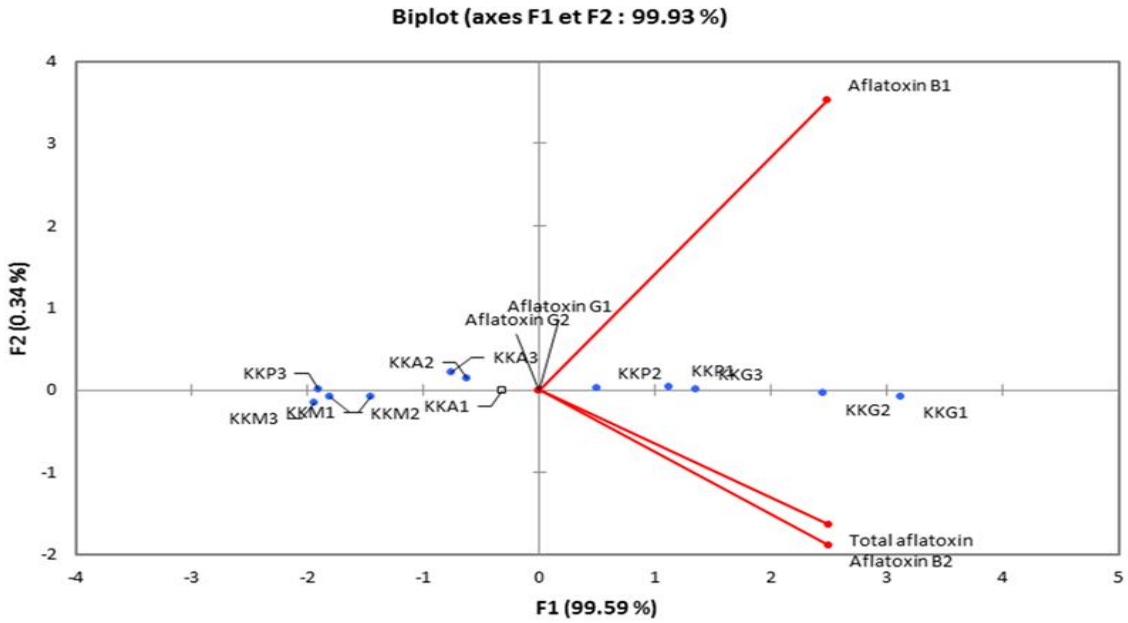

Figure-2. Principal component analyses according to aflatoxins content in samples.

Ascending hierarchical classification (AHC) gives a dendrogram grouping the analysed samples at two groups according to aflatoxins content Figure 3. The first group consisted of the samples KKA1, KKA2, KKA3, KKM1, KKM2, KKM3 and KKP3. The second group consisted of the samples KKG1, KKG2, KKP2, KKP1 and KKG3.

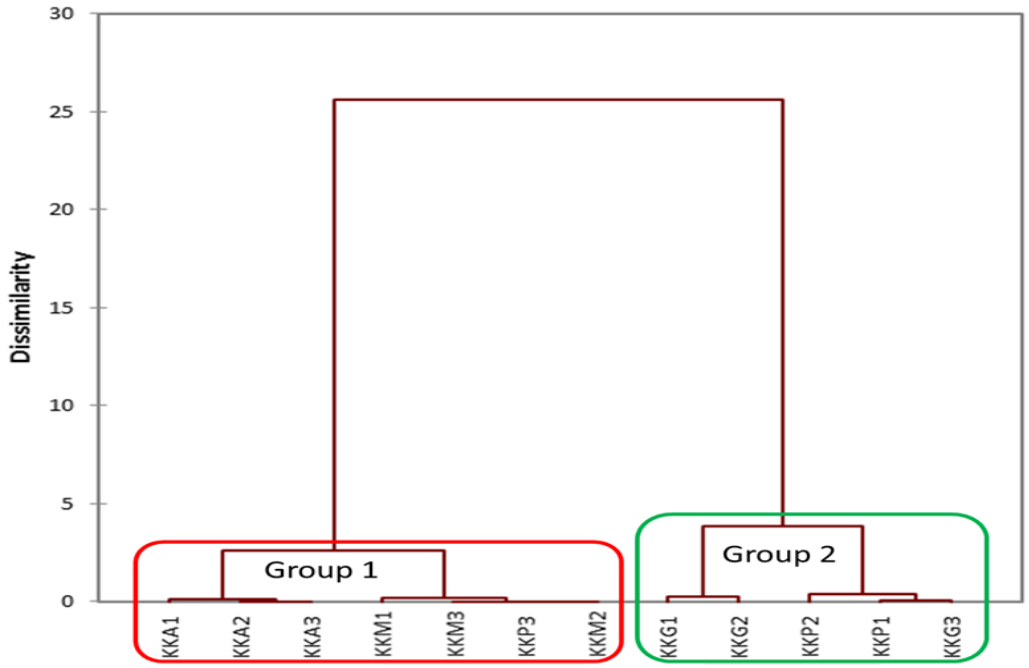

Figure-3. Ascending hierarchical classification according to aflatoxins content in samples.

3.5. Microbiological Results of Koura-Koura with Spices and Aromatic Leaves

Counts of different flora in the spice and aromatic leaf *koura-koura* samples values which are presented in Table 4. The total mesophilic aerobic flora (TMEF) ranged from 1.60 ± 1.57 x10^1 CFU/g (KKA3) to 3.70±1.93 x10^1 CFU/g (KKM1). Yeasts and moulds and coliforms (total and thermo-tolerant) were absent.

Table-4. Microbiological results of *koura-koura* with spices and aromatic leaves.

Sample	TMAF (CFU/g)	Yeasts and moulds (CFU/g)	Total coliforms (CFU/g)	Thermo-tolerant coliforms (CFU/g)
KKA1	2.50 ± 1.64 x10^1	ND	ND	ND
KKA2	2.10 ±1.48 x10^1	ND	ND	ND
KKA3	1.60 ± 1.57 x10^1	ND	ND	ND
KKP1	3.00 ± 1.86 x10^1	ND	ND	ND
KKP2	2.50 ± 0.28 x10^1	ND	ND	ND
KKP3	2.30 ± 1.69 x10^1	ND	ND	ND
KKG1	3.40 ± 0.82 x10^1	ND	ND	ND
KKG2	2.80 ± 0.24 x10^1	ND	ND	ND
KKG3	2.60 ± 1.98 x10^1	ND	ND	ND
KKM1	3.70 ± 1.93 x10^1	ND	ND	ND
KKM2	3.10 ± 0.43 x10^1	ND	ND	ND
KKM3	2.90 ±1.49 x10^1	ND	ND	ND
Threshold limit (2005/2073/CE)	10^6	10^4	10^3	10

Note: Legend: ND: Not detected.

4. DISCUSSION

The diagram used for *koura-koura* production in this study is a variation of the traditional diagram used by *koura-koura* producers Figure 1. The difference is in the rest period (5 h) after the addition of spice powders and aromatic leaves. The rest period reduces the microbial load and the aflatoxin level in the dough. The diagram was selected after several trials which allowed to control of the different parameters. Only the best parameters were selected and combined to give a good diagram.

The aflatoxin contamination occurred after the roasted seeds were ground into peanut paste. The contamination occurred at the public mill where the milling was carried out. Indeed, these public mills are not only

used to grind several products but are also poorly maintained (Garba et al., 2015). Mixing and de-oiling reduce the total aflatoxin content by more than 7%. As for the frying, it reduces the total aflatoxin content in *koura-koura* by more than 36%. Aflatoxin concentrations in *koura-koura* are lower than those found in *kluiklui* (25.54 to 455.22 µg/kg for AFB1 and 33.94 to 491.20 µg/kg for AFB2) by Adjou, Yehouenou, Sossou, Soumanou, and Souza (2012). Our values could be explained by the non-contamination of peanut seeds, the practice of good manufacturing practices.

According to their aflatoxin concentration, the samples are divided into two groups according to the ascending hierarchical classification. The first group includes samples KKA1, KKA2, KKA3, KKM3, KKP3, and KKM2. The second group includes samples KKG1, KKG2, KKP2, KKP1, and KKG3. The second group would be more concentrated in aflatoxin than the first. The principal component analysis pits aflatoxin B1 against total aflatoxin and aflatoxin B2. This would show that aflatoxin B1 is the main component and would represent the major part of total aflatoxin.

The use of spices and aromatic leaves in *koura-koura* resulted in a very significant reduction of aflatoxin B1, B2, and total aflatoxin concentration. Spices and aromatic leaves have a detoxification efficiency ranging from 17.06% (KKG1) to 74.62% (KKP3). The best reductions are observed with the 3% formulas for all spices and aromatic leaves. Pepper (average efficiency 74.62%) would have a very high detoxifying effect on aflatoxins followed by mint (average reduction 74.09%), garlic (average reduction 62.68%), and ginger (average reduction 37.66%). Indeed, the detoxifying properties of these spices have been widely studied by Goetz and Ghedira (2012) through free radical scavengers such as SAC and SAMC. Our values are significantly higher than those found (54.6% to 72.7%) by Olalekan-Adeniran, Adegoke, and Aroyeun (2016) who used a spice (*Aframumum danielli*) to reduce aflatoxins in *kulikuli*. This would be due to the composition of spices and their levels of anti-aflatoxigenic agents (antioxidants, vitamins, minerals).

Raw peanuts have a fairly high microbial load. Mejrhit, Taouda, and Aarab (2015) found TMAF values in peanuts ranging from $2.1x10^3$ CFU/g to $2.20x10^3$ CFU/g. Yeast and mould values ranging from $6x10^3$ CFU/g to $1.90x10^3$ CFU/g and coliform values ranging from $5.3x10^2$ CFU/g to $1.15x10^2$ CFU/g (Mejrhit et al., 2015). Our values are high for the TMAF and this could be explained by poor storage and poor preservation. Indeed, storage is done in dark rooms and on the ground. There is a significant decrease after roasting and de-oiling. The decrease in the number of sprouts in roasted peanuts is due to the increase in temperature (Garba et al., 2015). The heat during roasting would have destroyed total and thermos-tolerant coliforms. However, there is an appearance of yeast and mould flora and an increase in the TMAF in the peanut paste due to cross-contamination at the mill. Grinding at the public mill is a critical point to control in the production process. Spices and aromatic leaves have greatly contributed to reducing the total microbial load and eliminating fungi in *koura-koura*. These spices and aromatic leaves have antifungal and antibacterial activities that would decontaminate our samples. Indeed, several studies have shown the action of certain extracts, powders, and oils of spices and herbs such as ginger, garlic, cumin, chilli, onion, mace, coriander, cardamom, cinnamon, nutmeg, mint, laurel on microorganisms (Burt, 2004; Friedman, Henika, & Mandrell, 2002; Tajkarimi, Ibrahim, & Cliver, 2010; Tiwari et al., 2009). Microbiological analysis of *kluiklui* sold in Beninese markets revealed that the total mesophilic aerobic flora ranged from $4x10^4$ to $2x10^6$ CFU/g; total coliforms ranged from $1.0x10^2$ to $8.1x10^2$ CFU/g (Adjou et al., 2012). These results demonstrate strict adherence to GMP in our production, given the absence of coliforms and the low microbial load of our *koura-koura*.

5. CONCLUSION

The use of aromatic spices and leaves to reduce aflatoxins in *koura-koura* is a fairly simple, inexpensive technique with very little risk of toxicity. It is easily reproducible and is necessary to ensure the consumer safety. The production process of *koura-koura* has certain critical points that must be controlled by the HACCP method to

avoid cross-contamination. The information provided by this study would be of major importance in the control of aflatoxins and the risks they pose.

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