Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/12182
DOI URL: http://dx.doi.org/10.21474/IJAR01/12182

RESEARCH ARTICLE

STUDY OF AFLATOXINS IN CASHEW NUTS PRODUCED IN CÔTE D'IVOIRE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (LCHP)

M. Gouli[1], E. Zran[1], A. Yapi[2], A. Trokourey[1] and P. Drogui[3]

1. UFR-Sciences des Structures de la Matière et de Technologie, Université de Félix HOUPHOUET-BOIGNY. Laboratoire de Chimie Physique, 22 BP. 582, Abidjan 22 (Côte d'Ivoire).
2. Université LOROUGNON GUEDE de DALOA (Côte d'Ivoire). Laboratoire des Sciences et Technique de l'Environnement (LSTE), BP 150 Daloa (Côte d'Ivoire).
3. Institut National de la Recherche Scientifique. Département INRS-Eau Terre et Environnement, Université du Québec, 490 rue de la Couronne, Québec, QC G1K 9A9, Canada.

Manuscript Info

Manuscript History
Received: 20 October 2020
Final Accepted: 24 November 2020
Published: December 2020

Key words:-
Cashew Nuts, Aflatoxins, Aspergillus Molds, Limit of Detection, and HPLC

Abstract

The objective of this study is to isolate, identify and quantify four types of aflatoxins noted AFB1, AFB2, AFG1, and AFG2 that can be found in cashews grown in Côte d'Ivoire. These carcinogenic mycotoxins (AF) are secondary metabolite toxins produced by Aspergillus molds in plant foods.This work involved eleven (11) samples of 500 g of cashew nuts from eleven (11) cities of Côte d'Ivoire for the 2018-2019 campaign. These cities are: Béoumi, Bondoukou, Dabakala, Daloa, Douékoué, Ferkessédougou, Korhogo, Mignignan, Odienné, Sinématiali, and Touba. The test were carried out by high performance liquid chromatography (HPLC) after extraction of the four (4) mycotoxins on an immunoaffinity column at a flow rate of 3 mL / minute. These aflatoxins were identified and quantified from the following pairs of Retention time (Rt) in minutes and Limit of Detection (LD) in µg / kg: (13.777; 0.00143), (10.583 ; 0.00136), (9.901; 0.00151), and (8.184; 0.00564) respectively for AFB1, AFB2, AFG1, and AFG2. Our results show that all eleven (11) samples from these eleven (11) different cities contain aflatoxin (AFB1, AFB2, AFG2 and AFG1) contents below the national standard (2 µg / kg), that of the CODEX Alimentarius (1.4 µg / kg) and that of the European Union (2 µg / kg) indicating that cashews produced in Côte d'Ivoire comply with international standards and their consumption does not pose any risk to human health caused by the studied aflatoxins.

Introduction:-

Aflatoxins are mushrooms made up of 18 structurally close compounds, four of which are the most commonly encountered forms in food (AFB1; AFB2; AFG1; AFG2; AFM1; AFM2). In addition, the aflatoxins B1 and B2 are produced by Aspergillus flavus and the other four by Aspergillus parasiticus **(PACA 2008; Sevastianos et al., 2006)**. Among these aflatoxins, only aflatoxin B1 is the most common in food. These mushrooms are not very sensitive to most heat treatments of food (sterilization, pasteurization, freezing) or to drying (dehydration, freeze-drying) with the exception of roasting **(Afssa, 2009)**. It is directly linked to adverse health effects such as liver

Corresponding Author:-M. Gouli
Address:-UFR-Sciences des Structures de la Matière et de Technologie, Université de Félix HOUPHOUET-BOIGNY.Laboratoire de Chimie Physique, 22 BP. 582, Abidjan 22 (Côte d'Ivoire).

cancer and cirrhosis. Humans are exposed to aflatoxin from eating contaminated food **(Lama, 2008; André, 2007)** and have the most potent carcinogenic genotoxic properties **(Laure, 2006; Mamadou, 1978; Codex Alimentarius Commission, 2004)**.

In developing countries producers of cashew nuts like Côte d'Ivoire, aflatoxins are problematic for a public health. Indeed, it can happen that the cashew nut is contaminated by these aflatoxins in the fields, after harvesting and during storage. Producers of contaminated crops therefore suffer financial losses due to the rejection of their products, by the reduction of the value of said products on the market, the impossibility of accessing international trade and the trade restrictions imposed by international standards **(Eric, 2003)**.

The various studies carried out on aflatoxins aim to preserve the health of populations threatened by this type of mycotoxin. To find a solution to this public health problem, it is essential to assess the degree of contamination of cashew products and derivatives, to specify the impact of the consumption of cashew products contaminated by aflatoxins on human and animal health.

The objective of this study is to identify and quantify aflatoxins by high performance liquid chromatography (HPLC), in the cashews produced in Côte d'Ivoire in 2019.

Materials and Methods:-

Cashew nut:

Eleven (11) samples (500 g each) of cashew nuts from the 2019 harvest were provided by storage points (cooperatives) from eleven (11) different selected cities (Béoumi, Bondoukou, Dabakala, Daloa, Duékoué, Ferkessédougou, Korhogo, Minignan, Odienné, Sinématiali and Touba). Fifty-five (55) samples totaly were carried out, including 5 by region (11).Thus, the average of the results of the five tests obtained by region are recorded in the Table III.

Analysis protocol:

The analysis protocol used during this study is similar to that described by **Nadia (2011)**. The whole sample is ground and 20 g are taken and then mixed with 100 mL of extraction solvent composed of water / methanol (V / V). The solution obtained is centrifuged at 5000 rpm and homogenized, protected from light. The supernatant is filtered under vacuum on Wattman glassmicrofiber filters(GF/C circles 45 µm diameter) in an Erlenmeyer flask. 10 mL of this filtrate is diluted in 40 mL of a phosphate buffer solution (PBS) and then filtered on Wattman glass. The phosphate saline solution (PBS) of pH 7.3 is prepared by dissolving 0.2 g, 0.2 g, 1.16 g, and 8 g respectively of KCl, KH_2PO_4, Na_2HPO_4, and NaCl in one liter of distilled water **(Daradimos et al., 2000)**.

A volume (10mL) of the filtrate is loaded onto the immunoaffinity column at a flow rate of 3 mL / minute. In a hemolysis tube, there (immunoaffinity column) is applied 1.5 ml of methanol (the eluent). The column is dried completely by applying vacuum. The eluate is collected, diluted in 1.5 ml of distilled water and recovered in a 2 ml Vial for chromatographic analysis.

Analytical conditions at HPLC:

The chromatograms were recorded and analyzed using a DELL computer with LC solution software. The analytical conditions at HPLC are presented in Table I.

Table I:- Analytical conditions at HPLC.

Chromatograph	**Operating conditions**
Column	Shim pack VP-ODS, 250 L x 4.6 mm
Flowrate	0.5 mL/min
Mobile phase	Acetonitrile/Methanol (50/50, V/V)
Injected volume	20 µL
Detector	Flurescence
Analysis	25 min

Limit of detection (LD) and quantification (Logeais, 1986; wafa, 2013)

The LD was calculated according to (Eq. 1)

$$LD = 3,3 \times \frac{\sigma}{S} \quad (1)$$

σ is the standard deviation; S the slope of the calibration curve.

The limit of quantification (LQ) is defined as being 3.3 time the limit of detection (Jennifer, 2009) (Eq. 2).

LQ = 3,3 x LD (2)

Results and Discussion:-

Quantification of Aflatoxins by HPLC in Cashews

• Aflatoxin content in a contaminated reference sample

The quantitative aflatoxin analysis was performed on a reference sample of contaminated cashews. The chromatogram from the contaminated sample is shown in Figure 2

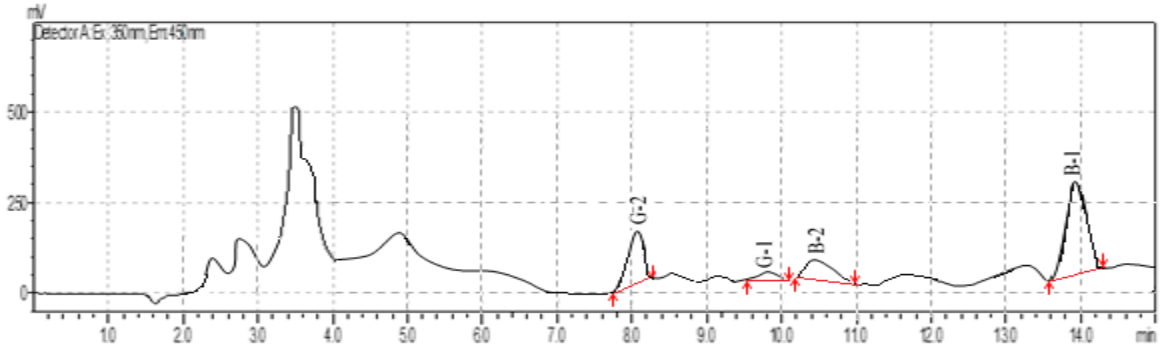

Figure 2:- Chromatogram of a contaminated reference sample.

The parameters resulting from the chromatogram are mentioned in Table II.

Table II:- Content of aflatoxins in a contaminated reference sample.

Retention time	Aflatoxins	Concentration (µg/kg)	Total aflatoxins (µg/kg)	Limit of detection (µg/kg)	Limit of quantification(µg/kg)
8.184	G-2	3.593		0.00564	0.01878
9.901	G-1	1.774	14.571	0.00151	0.0050
10.583	B-2	2.359		0.00136	0.0045
13.777	B-1	6.845		0.00143	0.0047

Aflatoxins content in cashew samples

Quantitative analysis of total aflatoxin (B1, B2, G1, G2) was performed on 11 cashew samples from 11 production cities. The results are reported in Table III.

Table III:- Aflatoxins levels in cashew samples.

Sample of	Retention time	Aflatoxins	Concentration (µg/kg)	Total aflatoxins (µg/kg)	Limit of detection (µg/kg)	Limit of quantification (µg/kg)
Beoumi	8.184	AFG2	0.065	0.1	0.00564	0.01878
	No peak	AFG1	ND		0.00151	0.0050
	10.583	AFB2	0.022		0.00136	0.0045
	13.777	AFB1	0.013		0.00143	0.0047
Bondoukou	No peak	AFG2	0.01878	ND	0.00564	0.0050
		AFG1				
		AFB2	ND		0.00151	0.0045
		AFB1			0.00136	0.0047
					0.00143	

Dabakala	No peak 9.901 LQ No peak 14.304 0.023	AFG2 AFG1 AFB2 AFB1	ND < ND 	0.00564 0.023	0.01878 0.00151 0.00136 0.00143	 0.0050 0.0045 0.0047
Daloa	8.184 0.065 No peak 10.583 13.777 0.013	 AFG1 AFB2 	AFG2 ND 0.022 AFB1 	 0.1	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Duekoué	7.979 No peak No peak No peak	AFG2 AFG1 AFB2 AFB1	0.0219 ND ND ND	0.0219	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Ferkessedougou	8.184 No peak 10.583 13.777	AFG2 AFG1 AFB2 AFB1	ND	ND	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Korhogo	8.184 LQ No peak LQ 10.583 13.777 LQ	AFG2 AFG1 AFB2 AFB1	 < ND <	ND	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Minignan	8.184 ND No peak 10.583 13.777 LQ	 AFG1 AFB2 AFB1	AFG2 ND ND <	ND	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Odienné	8.184 0.0219 No peak ND 10.583 13.777 ND	 AFB2 	AFG2 AFG1 ND AFB1 	0.0219	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Sinématialie	8.184 No AFG1 10.583 13.777	AFG2 peak AFB2 AFB1	 ND 	 ND 	0.00564 0.00151 0.00136 0.00143	0.01878 0.0050 0.0045 0.0047
Touba	No peak No peak No peak 14.060 0.013	AFG2 AFG1 AFB2 	ND ND ND AFB1 	0.00564 0.013	0.01878 0.00151 0.00136 0.00143	 0.0050 0.0045 0.0047

AF: Aflatoxins, ND: Not Detect

Results Analysis:-

In this analysis, we focus on aflatoxin B1 because it is the most often encountered in food and it is more toxic than other types of aflatoxins **(El Himer, 2017)**. So, among the samples analyzed, only 36.36 % (4 samples) shew the

detectable concentrations of aflatoxins B1 ranging from 0.013 to 0.023 µg / kg. According to **Gnonlonfin (Gnonlonfin et al., (2011))**, the presence of aflatoxin B1 is explained by the fact that hygienic control measures, adequate harvesting and collection methods, drying, sorting and storage are not well controlled. Aflatoxin B1 is also found in several plant foods.

However, the levels of all the aflatoxins (G2, G1, B1 and B2) obtained in the eleven (11) samples are lower than the national standard (2 µg / kg), CODEX Alimentarius (1.4 µg / kg), and European Union (2 µg / kg) **(Julien, 2013)**. Furthermore, the results from our testings are in the same order than those obtained by **José et al. (2015)** in cashew nuts from Mexico (0.02 µg / kg). Likewise, the results obtained are consistent with those reported by many authors in other foods. According to **Aissata et al., (2017)**, the analysis of 12 samples of peanut paste taken from the markets of three large municipalities, namely Yopougon, Abobo, and Adjamé, showed aflatoxin B1 contamination at levels as low as 2 µg / kg (authorized contamination limit). All these observations show that all samples analyzed have acceptable levels of aflatoxins for a safe consumption.

However, a higher aflatoxin B1 concentration was reported by **Paula et al., (2019)** in random samples of peanuts and cashews from city grocery and supermarket shelves from Brazil with an average content of 14.0 and 1.08 µg / kg respectively and also by **Gnonlonfin et al. (2011)** in cashew nuts produced in 04 departments from Benin (0.04 to 0.2 µg / kg).

Conclusion:-

This work helps to better understand the problem of aflatoxins in Côte d'Ivoire, in particular in the case of cashew nuts from the 2018-2019 campaign. The analyzes carried out by high pressure liquid chromatography (HPLC) on the eleven (11) averages of cashew samples from eleven (11) different cities available to us show that these samples contain aflatoxin contents (AFB1, AFB2; AFG1 and AFG2) lower than the national standard (2 µg / kg), CODEX Alimentarius (1.4 µg / kg), and European Union (2 µg / kg). Therefore, there is no risk for the consumption of cashew-based products from Côte d'Ivoire based on the level of aflatoxins Consequently, the cashew nuts from the 2018-2019 campaign meet international standards and can be exported to European and African markets.

Acknowledgements:-

The authors express their sincere thanks to the management and staff of the National Support Laboratory for Agricultural Development of Côte d'Ivoire (LANADA-CI) for their technical contribution to the realization of this work.

References:-

1. Agence Française de Sécurité Sanitaire des Aliments (Afssa). (2009). Evolution des risques liés à la présence desmycotoxines dans les chaînes alimentaires humaine et animale. P.13-20, Vol. 308.
2. Aissata D., Marc G. BI I., Dakmak K.N. et Jacques A. Y. (2017). Détermination de la contamination par l'Aflatoxine B1 de la pâte d'arachide consommée par la population en Côte d'Ivoire : intérêt de la Chromatographie sur Couche Mince.International Journal of Biological and Chemical Sciences. P. 1646-1654, Vol. 1654.
3. André EL KHOURY.(2007). Champignons Mycotoxinogènes et Ochratoxine A (OTA) et Aflatoxine B1 (AFB1) dans les vignobles libanais : occurrence et origine. P. 10- 18, Vol. 213.
4. Commission du codex alimentarius. Organisation des Nations Unies pour l'Alimentationet l'Agriculture. (2004). Document de travail sur les aflatoxines dans les fruits à coque (Autres que les amendes, les noisettes et les pistaches), y compris les informations complémentaires sur la contamination par les aflatoxines et sur les méthodes d'analyses permettant de détecter la présence des aflatoxines dans les fruits à coque. Rotterdam (Pays-Bas). P. 22-28.
5. Daradimos E., Marcaki P. and Koupparis M. 2000. Evaluation and validation of two fluorometric HPLC methods for the determination of aflatoxin B1 in olive oil. Food Addit. Contam. 17, 65– 73.
6. El Himer N. G. S.(2017). Etude mycologique et identification des souches fongiques toxinogènes isolées des amandes et arachides tlm. P. 46, Vol. 76.
7. Eric J. L.(2003). Les anacardiers, les noix de cajou et la filière anacarde à Bassila et du Benin. P. 16-17 et P. 56, Vol. 75.
8. Gnonlonfin G. J. B, Fanou L., Fandohan P., Adéoti R., Coulibaly O., Hell K., Dohou V. (2011). Amélioration et contrôle de qualité des produits agricoles alimentaires au Bénin. P.17, Vol. 79.

9. Jennifer W., Isabelle S., Jean François F., Pascal H. (2009). Dosage par chromatographie liquide haute performance de la luméfantrine sur spot de sang séché. Article original. P. 151, Vol. 154.
10. José A. G., Magda C. M., Francisco R. C., Silvia R. V. (2015). Aflatoxins in Walnut (Juglans regia L.), Pecan (Carya illinoinensis (Wangenh.) K. Koch) and Cashew (Anacardium occidentale L.) Nuts of Mexico. Pharmaceutica Analytica Acta P. 5.
Julien D.(2013).Profil de contamination des denrees alimentaires en aflatoxines et en histamine au Sénégal. P. 5 et P. 19.
11. Lama S.(2008). Evaluation scientifique des risques toxiques liés à certaines substances chimiques (Additifs Alimentaires) et contaminants (mycotoxines). P. 150-152, Vol. 224.
12. Laure M. N. (2006). Développement de dosages immunologiques par fluorescence perspectives pour l'élaboration d'un capteur en flux des agents de la menace. P. 62-65, Vol. 336.
13. Logeais M. (1986). Optimisation de la productivité des analytiques de contrôle d'un médicament. P. 21, Vol. 91.
14. Mamadou D.(1978). Etude des problèmes posés par les aflatoxines dans les aliments du bétail et de l'Homme. P. 2 et 17, Vol. 93.
15. NadiaA. (2010). Etude des populations du genre Aspergillus et Penicillium et de leurs mycotoxines isolées des épices et des légumes secs. P. 72, Vol. 110.
16. PACA : (Partenariat pour la lutte contre l'aflatoxine en Afrique). (2008). Atelier régional sur le défi de l'aflatoxine dans les Etats d'Afrique de l'Ouest-CEDEAO. P.4.
17. Paula K., Patrícia P. M., Ariadne N. A., Leonardo D. M., Mariana L. O., Walicyranison P. S. R., George Q. B., Guilherme M. C., Isarita M. (2019). Risk assessment of the occurrence of aflatoxin and fungi in peanuts and cashew nuts. Brazilian Journal of Pharmaceutical Sciences, P. 7
18. Sevastianos R., Nabila Z., Ghislane S., Abdelghafour T. E.(2006). Mycoflore naturelle des olives dans les maâsra et pouvoir toxinogène des souches d'Aspergillus sur céréales. P. 188, Vol. 192.
19. Wafa B., Mbarek A., Najib B. H. (2013). Optimisation et validation d'une méthode de dosage par HPLC/DAD d'un antihypertenseur de zofénopril. P. 48, Vol. 50.