

Full Length Research Paper

Investigation on aflatoxin M1 content of traditional cheese wagashi produced in Benin

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Mould growth on food is a common and recurring problem often leading to occurrence of mycotoxins inside foodstuffs. Mycotoxins are toxic metabolites known to induce serious health threats such as liver, kidney or nervous system damage, immunosuppression and carcinogenesis in animals and human. Aflatoxin M1 (AFM1) particularly is a carcinogenic metabolite often found in dairy products especially cheese. The aim of this study was to determine the presence of AFM1 in traditional cheese wagashi produced in Benin in order to evaluate possible hazards for consumers of this by-product largely appreciated and consumed by the populations in replacement of eggs and meat. It had consisted to collect fifty samples of wagashi near its retailers from seven markets in Benin and to analyze these samples for the detection of AFM1 by thin layer chromatography using aflatoxin M1 and Griseofulvin standards. Results obtained from this study showed that all samples analyzed were free of AFM1. However, all these samples contained other secondary metabolites which were not identified due to lack of references data and standards. Although samples analyzed were free of AFM1, the presence of other metabolites in cheese wagashi may pose serious health threats for consumers.

Keywords: Aflatoxin M1, moulds, cheese wagashi, Benin.

INTRODUCTION

For millennia, the presence of fungi in food has been both boon and bane to food stores. Fungi can spoil large quantities of food and produce inside dangerous toxins called mycotoxins that threaten human health (Dijksterhuis and Samson, 2007). Mycotoxins are toxic metabolites produced by some species of mould genera which invade foodstuffs and may grow on foods during storage under favorable conditions of temperature and humidity (Elkak et al., 2012). Of all mycotoxins, aflatoxins are a group of highly toxic metabolic products named as aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) and produced by fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus*

(Richard, 2007; Nilchian and Rahimi, 2012). They have been detected in various food commodities from many parts of the world and are presently considered as one of the most dangerous contaminants of food and feed (Hedayati et al., 2007). Until today, more than 300 aflatoxins have been detected and identified, the most toxic and diffuse is the AFB1, which has been classified as a group I carcinogen by the International Agency for Research on Cancer (IARC, 2002). AFB1 contamination can affect a variety of crops used as feed for dairy cattle. Once ingested, it is quickly absorbed and transformed into a hydroxylated metabolite which is secreted into the milk and has been designated as aflatoxin M1 (Anfossi et al., 2012). Aflatoxin M1 (AFM1) is an important hepatocarcinogen mycotoxin frequently found in milk and dairy products. Milk products such as cheese may be contaminated by aflatoxin M1 when dairy cattle have

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been fed with Aflatoxin B1-contaminated feeds (Dashti et al., 2009). The presence of AFM1 in cheese is thus considered undesirable due to toxic and carcinogenic properties (Kamkar, 2006; Amer and Ibrahim, 2010). Aflatoxin M1 is relatively resistant to heat treatments such as pasteurization of milk and to treatments used during cheese production (Anfossi et al., 2012). There are many reports on the occurrence of AFM M1 in commodities such as milk and cheese in several countries. To our knowledge, no study was conducted on Aflatoxin M1 content in cheese wagashi produced in Benin. It is therefore necessary to evaluate the presence of this toxic metabolite in traditional cheese wagashi obtained without refinement in Benin in order to assess the potential dangers for consumers of this foodstuff largely appreciated and consumed by Beninenses as substitute of meat and egg. This work is thus the first report on Aflatoxin M1 presence in local cheese wagashi samples marketed in Benin country.

MATERIAL AND METHODS

Sample collection

A total number of 50 commercial wagashi samples were collected for AFM1 analysis. All of the samples were randomly purchased from seven markets (Cotonou, Dassa-Zoume, Djougou, Gogounou, Kandi, Parakou and Pehunco) located in six agro-ecological zones of the eight in Benin from March 2013. The samples were placed into stomacher bag, sealed and transferred immediately to the laboratory and stored at 4°C in refrigerator until mycotoxins analysis.

Extraction of metabolites

Aflatoxin M1 extraction was performed by method described by Kamkar (2006). 15 g of cheese wagashi was homogenized in a stomacher bag with a stomacher apparatus for 2 minutes and treated three times with 50 ml of chloroform in a separating funnel. The chloroform extract was separated and dehydrated with anhydrous sodium sulfate and evaporated till dryness on water bath at 50°C under vacuum. The residues were dissolved in 1 ml chloroform before spotting on thin layer chromatography (TLC) plates.

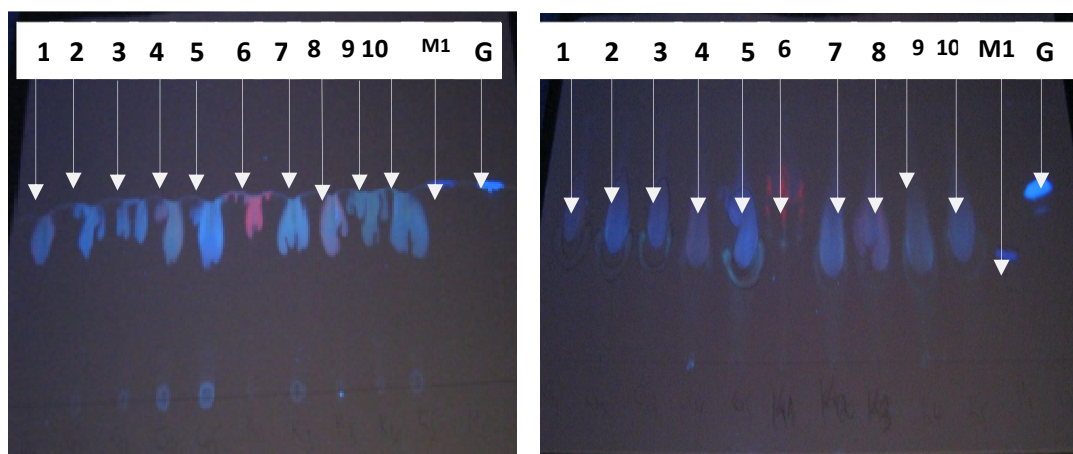
Detection of Aflatoxin M1

Thin layer chromatographic technique of the purified extract was done for the detection of aflatoxin M1 according to standards procedures described by Singh et al. (1991) and Kamkar (2006). Fifty micro liter chloroform extract was spotted on TLC plates (20 x 20 cm²) coated

with 0.25 mm thick silica gel (TLC Silica gel 60 F₂₅₄, Merck, Germany) with standards Aflatoxin M1 (10 µg/mL in acetonitrile, Lot: LB 96767; 46319-U, quantity: 1 mL, Exp: Nov/2015; SUPELCO Analytical society, Unity State of America) and Griseofulvin (Sigma, G4753-5G; 010M0537 Product of China, MSDS available SL 10243, EC. 204-767-4, WGK.3) and developed in the solvents system comprising TEF (Toluene/ethylacetate/Formic acid, 5:4:1 v/v/v) and CAP (Chloroform/acetone/2-propanol, 85:15:20, v/v/v). The retention factors (R_F^{CAP} and R_F^{TEF}) of the individual spots on TLC plates were calculated and compared with that of standard Griseofulvin (R_f^g TEF: 1.00; R_f^g CAP: 1.00) and the colour of each spot was compared with those of standard mycotoxins to aid in the identification of mycotoxins presented. Aflatoxin M1 was detected by visual examination of TLC plates under UV lamp at 365 nm and comparison of the fluorescent band with that of the standard aflatoxin M1 at 1 µg/mL.

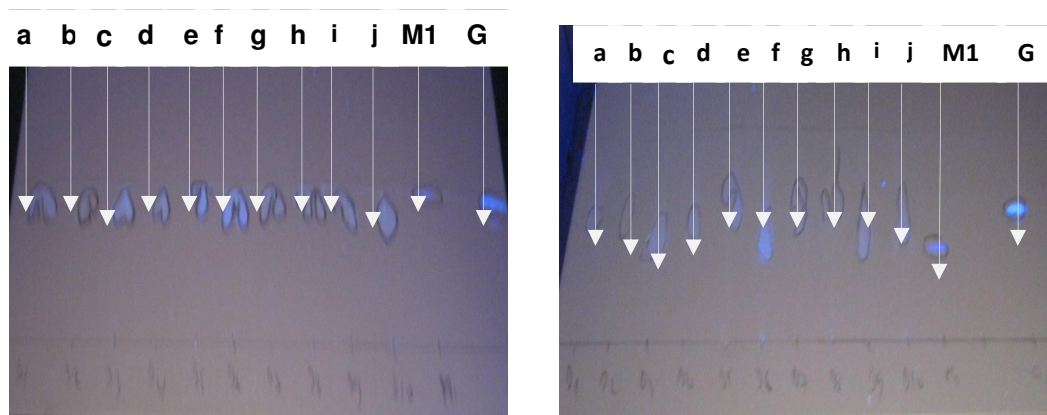
RESULTS AND DISCUSSION

Food safety and foodborne diseases constitute a growing public health problem (WHO, 2009). Thus, great attention must be given to food control especially mycotoxins investigation in food due to their deleterious effect on human health. Mycotoxins are metabolites of fungi capable of having acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic, and oestrogenic effects in man and animals (van Egmond et al., 2007). Aflatoxin M1 (AFM1) particularly is the principal hydroxylated metabolite of aflatoxin B1 excreted in milk, and subsequently it can be found in a large variety of dairy products such as cheese thus posing a potential risk to human health when consuming these products (Barug et al., 2004). Considering its significant impact on human health, investigation on AFM1 content in dairy products such as cheese wagashi becomes imperative (Elkak et al., 2012). The present study has investigated, for the first time, AFM1 content in traditional cheese wagashi processed in Benin. The results obtained from this work are presented in figures 1-2 and showed that all samples of wagashi analyzed were free of Aflatoxin M1. However, other metabolites not identified due to lack of references data and standards were noted. The absence of AFM1 in samples of wagashi could be explained by the fact that feed consumed by animals from which milk was extracted for the production of wagashi were not contaminated by *Aspergillus* species producers of Aflatoxin B1. Also, this absence of AFM1 could be linked to the very low contamination by Aflatoxin M1 of milk used for wagashi production. The non detection of AFM1 in cheese wagashi could equally be due to the non contamination of dairy cattle feeds by Aflatoxin B1 or by the non contamination of wagashi by moulds producers of Aflatoxins B1, B2, G1 and G2. Indeed, according to El Khoury et al. (2011) and Ertas et al. (2011),



Key: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 are spots of metabolites detected in samples, G: spot of griseofulvin standard, M1: spot of Aflatoxin M1 standard

Figure 1. Photography of spots of secondary metabolites extracted from wagashi samples collected in Kandi and Gogounou markets observed at UV 365 nm after TLC assay in solvents CAP (left) and TEF (right)



Key: a, b, c, d, e, f, g, h, i, j are spots of metabolites detected in samples, G: spot of standard griseofulvin, M1: spot of standard Aflatoxin M1

Figure 2. Photography of spots of secondary metabolites extracted from some wagashi samples collected in Dassa market observed at UV 365 nm after TLC assay in solvents CAP (left) and TEF (right)

aflatoxin B1 is hydroxylated into aflatoxin M1 in the liver of humans and other mammals that consume a diet contaminated with aflatoxin B1 leading to the accumulation of AFM1 in milk and consequently in cheese. Elkak et al. (2012) reported that the occurrence of AFM1 in cheese may be due to AFM1 contamination of raw milk used in cheese manufacture and synthesis of aflatoxins by *A. flavus* and *A. parasiticus* growing on cheese. Moreover, the non detection of AFM1 in wagashi could be explained by the fact that the process utilized for wagashi production allows the elimination of more Aflatoxin M1 in whey avoiding its presence in this by-product at detectable level by thin layer chromatography. Also, the conditions and methods of preservation of

wagashi by retailers allow the elimination of part of this metabolite from wagashi. In fact, it has been demonstrated that the concentration of AFM1 in cheese can also depend on the technology used in the production process, on the type of cheese and on the water content in the final product (Montagna et al., 2008). In addition, according to Ertas et al. (2011), the storage conditions of products including humidity and temperature are also important for toxin production in cheese. Once more, the absence of AFM1 in cheese wagashi could be linked to the fact that the detection limit of Aflatoxin M1 by thin layer chromatography is high whereas aflatoxin M1 content of this cheese is low. Indeed, according to Touchstone (1983), thin layer

chromatography's disadvantage is that its detection limit is a lot higher. In sum, AFM1 incidence of cheese wagashi samples analyzed in this study was null contrary to several studies in different countries performed by Kamkar (2006), Tekinsen and Ucar (2008), Aygun et al. (2009), Dashti et al. (2009) and Manetta et al. (2009) who have reported high or low contamination levels of AFM1 in different categories of cheese samples. These significantly variation of AFM1 content in cheese wagashi with results from other studies may be explained in part by various influencing factors including different contamination levels in milk, cheese manufacturing procedures and storage types of cheese, conditions of cheese ripening, analytical methods and finally the geographical and seasonal effects (Galvano et al., 1996; Oruc et al. 2006, Deveci, 2007).

The presence of other metabolites in cheese wagashi in large quantity because of the high brightness and large space of their spot compared to standards may pose health treats to consumers. These metabolites merit to be identified and quantified and whether harmful on human health must be regulated by regulatory agency in Benin.

Although the size of samples studied was weaker and results obtained from this study may not reflect inevitably the real results on all samples produced in Benin at all seasons, this work gives informations about the incidence of Aflatoxin M1 and other secondary metabolites in cheese wagashi. Study may be taken back on a large scale of wagashi samples in all seasons of the year in the meantime defined with appropriate and modern techniques such as High Performance Liquid Chromatography.

CONCLUSION

Aflatoxin M1 contamination of dairy products such as cheese appear to be a potential public health hazard for consumers. The present study had assessed the presence of AFM1 in cheese wagashi and showed that although all samples analyzed contained other secondary metabolites not identified, they were free of AFM1. Further studies are needed to be performed on the mycotoxins contents of wagashi by modern techniques such as HPLC in order to inform consumers about the potential health consequences of mycotoxins detected and to help to the regulations of these toxins in cheese wagashi in our country Benin.

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