

Full Length Research Paper

Fate of aflatoxins during traditional melon cake and sauce processing

Chibundu N. Ezekiel^{1,2*}, Yinka M. Somorin³, Michael Sulyok² and Rudolf Krska²

¹Department of Microbiology, School of Science and Technology, Babcock University, Ilishan Remo, Ogun State, Nigeria.

²Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad Lorenzstr 20, A-3430 Tulln, Austria.

³Microbiology Department, School of Natural Sciences, National University of Ireland, Galway, Ireland.

Received 27 December, 2016; Accepted 4 April, 2017

Melon cake is a non-fermented, traditionally processed food product obtained from melon seeds, and it is consumed as a snack or added to soup. Melon sauce is obtained during the final step of melon cake processing and it is used with melon cake during soup making. This study focused on evaluating the fate of aflatoxin during traditional melon seed processing into melon cake and sauce as a combined product. Shelled melon seeds were purchased from local markets and traditionally processed into melon cake and sauce. Samples were obtained at each processing step and analysed for aflatoxins by LC-MS/MS. Aflatoxin B₁ (AFB₁), AFB₂, AFG₁ and total aflatoxin (sum of AFB₁, AFB₂ and AFG₁) levels in the starting raw material (melon seeds: 39.5, 3.5, 1.97 and 44.9 µg/kg) were reduced by 95, 82, 85 and 94%, respectively, in the finished product (boiled melon cake and sauce) ready for direct consumption. The traditional process of making melon cake and sauce reduced aflatoxin levels below the regulated limits and may lower aflatoxin exposure among melon consumers.

Key words: Aflatoxin, consumer health, food processing, food safety, melon cake, melon sauce.

INTRODUCTION

Melon (*Colocynthis citrullus* L.) seed is a highly nutritious oil seed consumed widely in Nigeria and across West Africa. It contains more than 50% oil and is rich in essential amino acids, vitamins and micronutrients (Akobundu et al., 1982). Melon seeds and their products

are very susceptible to infection by aflatoxigenic fungi due to high ambient temperature and relative humidity during storage and hence aflatoxin contamination (Bankole et al., 2004a, b, 2006, 2010; Ezekiel et al., 2016; Somorin et al., 2016), and may possibly increase

*Corresponding author. E-mail: chaugez@gmail.com.

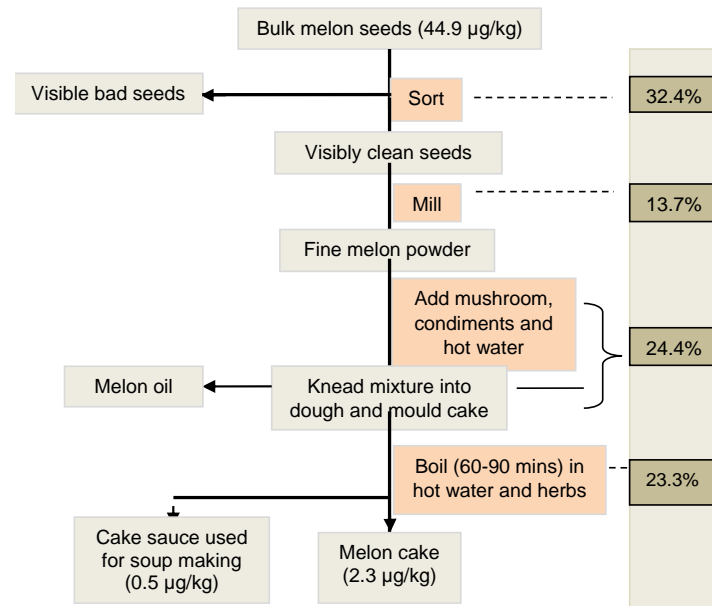


Figure 1. Flow chart for the production of melon cake and sauce with the resulting percentage total aflatoxin reduction. $\mu\text{g}/\text{kg}$ = total aflatoxin concentrations in foodstuff: Inputs, processes and products Processing interventions Percentage reduction of total aflatoxins by intervention step.

exposure to aflatoxin among its consumers. Aflatoxin exposure has been associated with liver cancer, immune system suppression and growth retardation in humans (especially children) (Gong et al., 2002, 2012; Turner et al., 2003, 2007). In addition, aflatoxin contamination of melon seed restricts its trade, especially to the European Union where this foodstuff is highly sourced as an alternative oilseed to groundnut. This high demand of melon seeds both locally in Nigeria and internationally, and the aflatoxin menace it faces continues to arouse interest on possible exposure control measures among its consumers. While there are several strategies to control exposure to aflatoxins, traditional processing has been shown to be simple and effective for aflatoxin reduction in some foodstuffs (Karlovsky et al., 2016).

Melon seeds are traditionally processed into several products such as melon ball snack 'robo', 'egusi' soup and the fermented condiment 'ogiri' (Bankole et al., 2010). Melon cake is a non-fermented, traditionally processed product from melon seeds in Nigeria and some parts of West Africa. It is a common snack (Ajuru and Okoli, 2013) for some tribes during traditional meetings and is mainly used as substitute for animal protein sources (e.g. meat and fish) during the preparation of "egusi" soup. The processing scheme for melon cake from shelled melon seeds is shown in Figure 1. Melon sauce is a side product obtained during the final step of melon cake processing and is mainly used for

soup making. Traditional food processing (e.g. hand sorting and fermentation) has been shown to significantly reduce mycotoxin (e.g. aflatoxin) levels in maize during processing by up to 96 to 99% and 61 to 83%, respectively (Matumba et al., 2015; Okeke et al., 2015). Roasting groundnut and fermentation of *Aspergillus flavus*-contaminated melon seeds to *ogiri* were shown to reduce aflatoxin levels by about 10 times and 100%, respectively (Afolabi et al., 2015; Ogunsanwo et al., 1989). In spite of the wide consumption of melon cake and the reports of high mycotoxin contamination of melon seeds, there is limited evidence on the effects of traditional processing on mycotoxin levels in melon seed-based foods. As such, this study aimed to investigate the fate of aflatoxins during the processing of melon cake so as to determine whether traditional processing of melon seeds to melon cake and sauce reduces aflatoxin levels.

MATERIALS AND METHODS

Samples

Shelled melon seeds were purchased from local urban markets where this crop is sold in large quantities in Lagos State, Nigeria in September 2014. The bulk melon sample weighed about 1 kg. The sample was traditionally processed into melon cake by a frequent producer and consumer of the product according to Figure 1. Samples (10 g each) for analysis were collected from each of the

Table 1. Aflatoxin levels from raw materials (melon seeds) to their products (melon cake and sauce) and their percentage reduction.

Materials	Concentrations ($\mu\text{g}/\text{kg}$)			
	Aflatoxin B ₁	Aflatoxin B ₂	Aflatoxin G ₁	Total aflatoxin ^a
Melon input ^b	39.5	3.5	1.97	44.9
Mushroom input ^c	–	–	–	–
Cake sauce	0.125 ^f	0.2 ^f	0.15 ^f	0.48
Melon cake	1.74	0.43	0.15 ^f	2.32
Melon cake + sauce ^d	1.87	0.63	0.3	2.8
Aflatoxin reduction (%) ^e	95.3	82.0	84.8	93.8

^aTotal aflatoxins: Σ aflatoxin B₁ (AFB₁), AFB₂ and AFG₁; ^bAflatoxin level in bulk melon/raw material; ^cAflatoxin level in mushroom; no aflatoxin detected; ^dSum of aflatoxin levels in melon cake and sauce because of their combined usage in soup preparation; ^eOverall percentage reduction of aflatoxin levels due to processing; ^fValues are LOD/2.

following stages in triplicates: bulk melon seeds, visibly bad (mouldy, discoloured and unhealthy-looking) seeds were removed, fine melon powder obtained from visible clean seeds, mushroom (bought from local market) added to thicken/bind the melon dough during the kneading step, molded melon cake obtained from the kneading process, cake sauce obtained after the boiling process, and the final product (boiled melon cake). The triplicate samples were composited and quartered to obtain representative samples for liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis. All samples were ground to powder and along with cake sauce, were kept at -20°C prior to analysis.

Determination of aflatoxin levels in food samples

Five grams of each representative sample were analyzed for the presence of aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) by LC-MS/MS. The LC-MS/MS method including extraction and chromatographic separation details is as described by Malachova et al. (2014). Spiking and recovery details are as reported by Ezekiel et al. (2016).

Data analysis

Data analysis was performed using SPSS[®] 17.0 (Windows version, SPSS, IL, USA). The percentage reduction of toxins from raw material to finished product was calculated.

RESULTS AND DISCUSSION

Aflatoxins B₁, AFB₂ and AFG₁ were the only quantified aflatoxins in samples obtained from each stage during the processing of melon seeds into melon cake (Table 1). AFG₂ was not detected in the melon seeds used in this study unlike previous studies (Ezekiel et al., 2016; Somorin et al., 2016). The concentrations of aflatoxins in the starting material (melon seeds) and processed products as well as the overall reduction in aflatoxin types at the end of processing are given in Table 1. The levels of aflatoxins [AFB₁ (39.5 $\mu\text{g}/\text{kg}$), AFB₂ (3.5 $\mu\text{g}/\text{kg}$) and

AFG₁ (1.97 $\mu\text{g}/\text{kg}$) and total aflatoxins (sum of AFB₁, AFB₂ and AFG₁; 44.9 $\mu\text{g}/\text{kg}$)] in raw melon seeds were significantly reduced by the various processing steps applied in the food processing. As indicated in Figure 1, hand sorting of visibly bad seeds reduced the total aflatoxin level in the bulk melon seed by 32.4%, while milling the seeds further reduced the total aflatoxin levels by 13.7%. The findings, which agree with previous reports on the effectiveness of sorting and milling in aflatoxin/mycotoxin reduction in maize (Bullerman and Bianchini, 2007; Matumba et al., 2015), confirm that these two processes are critical first-line measures towards the reduction of aflatoxin exposure during food processing. Perhaps, the substitution of automated sorting techniques equipped with UV/fluorescent sensors for hand sorting, which may be most feasible with up-scaling of this traditional melon seed processing method, may further reduce the toxin content in the starting material.

The absence of aflatoxins in the mushroom samples used during the preparation of melon cake confirms our previous report, which showed no regulated mycotoxins were present in mushrooms (Ezekiel et al., 2013). The addition of mushroom (a thickener) and condiments (for flavour and aroma) followed by a kneading step, which results in extracted oil, yielded a further reduction of 24.4% of the total aflatoxin level in the starting material. The toxin loss is most likely shared between the oil extract (Bordin et al., 2014) which we could not analyse and the additives (condiments). Many condiments (e.g. spices) have shown promise to reduce aflatoxin levels in food materials- e.g. the essential/volatile oils of turmeric were reported to decrease AFB₁ and AFB₂ levels by 99.9 and 99.6%, respectively (Ferreira et al., 2013). A further 23.3% reduction was recorded after the extended cooking (boiling) of the moulded cakes for 60 to 90 min, to leave a final 2.3 and 0.5 $\mu\text{g}/\text{kg}$ total aflatoxin levels in the melon cake and cake sauce, respectively. This kind

of extended cooking in the presence of liquid which contains spices may have resulted in the toxin decrease; a fact that agrees with the suggestions of Park et al. (2005) and Park and Kim (2006) for moderate reduction of aflatoxins under normal cooking and pressure cooking of rice. Overall and in view of the fact that the cake and sauce are usually combined in the soup making process, the percentage reduction for the aflatoxins influenced by the entire processing method was appreciably high (82 to 95% for AFB₁, AFB₂ and AFG₁; 94% for total aflatoxins) (Table 1). Traditional processing of melon seeds, which had AFB₁ and total aflatoxins levels several times higher than the European Union regulated limits of 2 and 4 µg/kg, respectively, reduced aflatoxins levels below the regulated limits in the final products (melon cake and sauce) (AFB₁ = 1.87 µg/kg; total aflatoxins = 2.8 µg/kg). This suggests that consumption of melon cake and sauce may not pose a high risk of exposure to aflatoxins.

Conclusion

Melon seeds are highly prone to aflatoxin contamination and, this study has shown that traditional processing of melon seeds to melon cake and sauce is able to reduce aflatoxin levels and may consequently reduce aflatoxin exposure among melon seed consumers. In view of the overwhelming toxicological evidence available for aflatoxins in literature and the increasing exposure evident in sub-Saharan Africa, which cannot be attributed only to maize and groundnut (Abia et al., 2013; Ezekiel et al., 2014), it is recommended that aflatoxin mitigation approaches to lower human exposure via consumption of contaminated foods be adopted, from the pre-harvest through post-harvest to the food processing stage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Abia WA, Warth B, Sulyok M, Krska R, Tchana A, Njoh PB, Turner PC, Kouanfack C, Eyongetah M, Dutton MF, Moundipa PF (2013). Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. *Food and Chemical Toxicology* 62:927-934.
- Afolabi CG, Ezekiel CN, Kehinde IA, Olaolu AW, Ogunsanya OM (2015). Contamination of groundnut in south-western Nigeria by aflatoxigenic fungi and aflatoxins in relation to processing. *Journal of Phytopathology* 163:279-286.
- Ajuru MG, Okoli BE (2013). The morphological characterization of the melon species in the family Cucurbitaceae Juss., and their utilization in Nigeria. *International Journal of Modern Botany* 3:15-19.
- Akobundu ENT, Cherry JP, Simmons JG (1982). Chemical functional, and nutritional properties of egusi (*Colocynthis citrullus* L.) seed protein products. *Journal of Food Science* 47:829-835.
- Bankole SA, Adenusi AA, Lawal OS, Adesanya OO (2010). Occurrence of aflatoxin B1 in food products derivable from "egusi" melon seeds consumed in southwestern Nigeria. *Food Control* 21:974-976.
- Bankole SA, Lawal OA, Adebajo A (2004a). Storage practices and aflatoxin B1 contamination of "egusi" melon seeds in Nigeria. *Tropical Science* 44:150-153.
- Bankole SA, Ogunsanwo BM, Mabekoje OO (2004b). Natural occurrence of moulds and aflatoxin B1 in melon seeds from markets in Nigeria. *Food and Chemical Toxicology* 42:1309-1314.
- Bankole SA, Ogunsanwo BM, Osho A, Adewuyi GO (2006). Fungal contamination and aflatoxin B1 of 'egusi' melon seeds in Nigeria. *Food Control* 17:814-818.
- Bordin K, Sawada MM, Rodrigues CEC, da Fonseca CR, Oliveira CAF (2014). Incidence of aflatoxins in oil seeds and possible transfer to oil: a review. *Food Engineering Reviews* 6:20-28.
- Bullerman LB, Bianchini A (2007) Stability of mycotoxins during food processing. *International Journal of Food Microbiology* 119:140-146.
- Ezekiel CN, Sulyok M, Frisvad JC, Somorin YM, Warth B, Houbraken J, Samson RA, Krska R, Odebo AC (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology* 162(3):231-236.
- Ezekiel CN, Sulyok M, Somorin Y, Odutayo FI, Nwabakee SU, Balogun AT, Krska R (2016). Mould and mycotoxin exposure assessment of melon and bush mango seeds, two common soup thickeners consumed in Nigeria. *International Journal of Food Microbiology* 237:83-91.
- Ezekiel CN, Warth B, Ogara IM, Abia WA, Ezekiel VC, Atehnkeng J, Sulyok M, Turner PC, Tayo GO, Krska R, Bandyopadhyay R (2014). Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers. *Environment International* 66:138-145.
- Ferreira FD, Kemmelmeier C, Arroeteia C C, da Costa CL, Mallmann C A., Janeiro V, Ferreira F M, Mossini SA, Silva EL, Machinski M (2013). Inhibitory effect of the essential oil of *Curcuma longa* L. and curcumin on aflatoxin production by *Aspergillus flavus* Link. *Food Chemistry* 136:789-793.
- Gong YY, Cardwell K., Hounsa A, Egal S, Turner PC, Hall AJ, Wild CP. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo, West Africa: cross sectional study. *British Medical Journal* 325:20-21.
- Gong YY, Wilson S, Mwatha JK., Routledge MN, Castelino JM, Zhao B, Kimani G, Kariuki HC, Vennervald BJ, Dunne DW, Wild CP (2012). Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. *Environmental Health Perspectives* 120:893-896.
- Karlovsy P, Suman M, Berthiller F, De Meester J, Eisenbrand G, Perrin I, Oswald IP, Speijers G, Chiodini A, Recker T, Dussort P (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research* 32:179-205.
- Malachová A, Sulyok M, Beltrán, E, Berthiller F, Krska R. (2014). Optimization and validation of a quantitative liquid chromatography–tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. *Journal of Chromatography* 1362:145-156.
- Matumba L, Van Poucke C, Njumbe Ediage E, Jacobs B, De Saeger S. (2015). Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food Additives & Contaminants: Part A* 32:960-969.
- Ogunsanwo BM, Faboya O, Idowu O, Ikotun T, Akano DA (1989). The fate of aflatoxins during the production of "Ogiri", a West African fermented melon seed condiment from artificially contaminated seeds. *Molecular Nutrition & Food Research* 33(10):983-988.
- Okeke CA, Ezekiel CN, Nwangburuka CC, Sulyok M, Ezeamagu CO, Adeleke RA (2015). Bacterial diversity and mycotoxin reduction during maize fermentation (steeping) for ogi production. *Frontiers in Microbiology* 6:1402.
- Park DL, Lee C, Kim YB (2005) Fate of aflatoxin B1 during the cooking of Korean polished rice. *Journal of Food Protection* 68:1431-1434.
- Park JW, Kim YB (2006) Effect of pressure cooking on aflatoxin B1 in rice. *Journal of Agricultural and Food Chemistry* 54:2431-2435.

- Somarin Y, Akinyemi A, Bertuzzi T, Pietri A (2016). Co-occurrence of aflatoxins, ochratoxin A and citrinin in "egusi" melon (*Colocynthis citrullus* L.) seeds consumed in Ireland and the United Kingdom. *Food Additives & Contaminants: Part B* 9(3):230-235.
- Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives* 111:217-22.
- Turner PC, Collinson AC, Cheung YB, Gong YY, Hall AJ, Prentice AM, Wild CP. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology* 36:1119-1125.