

Full Length Research Paper

Effect of different cooking methods on aflatoxin fate in peanut products

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Peanut is still a major oilseed and staple crop in Senegal. It is, however, prone to contamination by aflatoxins, a major public health concern. This work was conducted to assess aflatoxin contamination risk linked with consumption of peanut-based dietary products and the influence of different cooking processes on aflatoxin fate. A survey made in the markets of Thies (Senegal) showed that the "second choice peanut" had the highest aflatoxin content with 50% samples above the 10 ppb limit set by EU for human consumption. First choice peanut contained lower concentrations of aflatoxin with all samples below the safe level of 10 ppb. Peanut flour and peanut butter, in spite of testing positive to aflatoxins (100%), had safe levels whereby butter was the least contaminated. A total reduction of aflatoxin level of about 82.5% was obtained when peanut was submitted to roasting, made into peanut butter, and further steamed. Steaming as well as boiling caused a reduction of aflatoxin level, but was however not significant.

Keywords: Aflatoxins, peanut, cooking methods, Senegal.

INTRODUCTION

Peanut plays a key role in the agricultural economy and represents an important source of income for rural farmers in Senegal. Peanut production is the backbone of an intense commercial and industrial activity for kernels, oil as well as staple human food and animal feedstuff. It is consumed in various forms such as raw kernels, flour and also after roasting in the form of snacks and peanut butter or after incorporation in many frequently eaten local dishes. For some dishes like "sombi guerte", rice is pound together with the peanut flour and subjected to boiling in water for about 30 min. "Mbaxal", a very popular dish in Senegal, is prepared by adding peanut flour onto the almost cooked rice, millet or sorghum to be steam cooked for about 15 min. Peanut kernels undergo roasting for the preparation of "ngalakh" a sweet mixture of peanut butter and baobab fruit juice. For "mafe" – a popular dish in West Africa – and "dakhin" – another Senegalese speciality – the peanut butter is subjected to additional boiling together with the adequate ingredients.

Peanut and its products are prone to contamination by aflatoxins known to be a potent human carcinogen (Wild and Turner 2002). Aflatoxins primarily produced

by *Aspergillus* section Flavi remain the most dangerous mycotoxin in the world, recognized as a cause of liver cancer and various additional important toxic effects (Williams et al., 2004). In West African countries, mainly in rural areas, exposure to aflatoxin occurs throughout the whole life resulting in growth faltering associated with high blood aflatoxin-albumin adducts and cancers (Egal et al., 2005).

In Senegal, where peanut is widely grown and consumed in a daily basis, the frequent occurrence of drought and scarce rains, added to inadequate production practices, may be conducive to heavy field infestation by *Aspergillus flavus* (Arunyanark et al., 2009). There is an increased susceptibility to *A. flavus* infection in immature and damaged peanut kernels. These two kinds of peanuts are frequently those kept for consumption by the peanut-farming households because of their low market value, thus resulting in an increased aflatoxin exposure risk (Diouf et al., 2005).

The current mycotoxin issue in developing countries faces food safety challenges. Managing mycotoxin exposure in developing countries with widespread rural poverty is a food quality issue that must be balanced by food security considerations. The highest rate of hepatocellular carcinoma prevails in the Peanut Basin in Senegal, where peanut is eaten in various forms in a daily basis. In rural African conditions, peanut con-

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sumption is often linked with exposure to high aflatoxin level (Odoemelam and Osu, 2009). For example, 40% of artisanal peanut butter sold in different markets in Dakar contained B1 aflatoxin levels well above 4 ppb (Diop et al, 2000). In the Peanut Basin where peanut is the backbone of the agro-economic activity and a main staple food, 80% of locally made oil was shown to contain aflatoxin B1, B2, G1 and G2. Aflatoxin B1 occurred in 85% of the samples with about an average content of 40 ppb (Diop et al, 1999).

This work was conducted to find out in which extent peanut cooking methods used in Senegal could be efficient in reducing aflatoxin content of food.

MATERIALS AND METHODS

Peanut products from the markets

Samples of peanut kernels, flour and peanut butter were collected from different markets in Thies, Senegal (latitude 14° 48' 0.00" N, longitude 16° 55' 58.80" W) in June 2007. A total of 20 samples of 1 kg each of "first choice" and "second choice" kernels were collected. The "first choice" peanut kernels are good quality sorted seeds, mature and healthy looking while the "second choice" peanut is made of a mixture of immature seeds, damaged kernels split cotyledons and kernels without seed coats for the most part. In addition, peanut flour ("Noflaye") and peanut butter ("Dégué") were sampled once (20 samples of 500 g each).

Inoculation of peanut seeds with *A. flavus*

Three portions of 3 kg first choice peanut kernels with 0.28 ng/g aflatoxin content were inoculated with a spore suspension of the toxigenic *A. flavus* L strain M12-12. Inoculation was performed by injecting 50 mL of a spore suspension of *A. flavus* adjusted to 10^6 spores/mL into 3 kg sample of first choice peanut contained in 10 L plastic bag. Samples were then incubated at room temperature (28-30 °C) in the laboratory for 5 days.

Influence of cooking methods on aflatoxin fate

Different cooking methods used for peanut based dishes were tested for aflatoxin fate contained in kernels before processing. The cooking methods comprised steaming peanut flour, boiling of flour, and roasting kernels followed by grinding into peanut butter and subsequent boiling.

Aflavus M12-12 inoculated peanut was submitted to different cooking methods. Aflatoxin was performed on the non-inoculated control and inoculated samples as well as during cooking.

Peanut flour steaming test

Peanut flour was introduced into a couscous steamer set above a cooking pot containing 2 L of distilled water and heated on a hot plate. Aflatoxin was assayed at 15 min intervals, starting upon onset of vapors. This procedure was repeated 3 times.

Peanut flour boiling test

Peanut flour (500 g) and water were mixed in a cooking pot and boiled on a hot plate. An initial aflatoxin analysis was made 30 min after onset of the first vapors. This assay was followed by a 30 min sampling interval to monitor changes in aflatoxin content.

Test on peanut butter

The preparation of peanut butter involves dry roasting of first class peanut seeds followed by removal of shells and grinding of the seeds to obtain butter. First class peanut samples inoculated with *Aspergillus flavus* and non inoculated control samples were dry roasted in an oven at 140°C for 1h. Similar roasting conditions were found adequate to obtain good quality peanut butter. A subsample of 500 g peanut butter was submitted to water cooking in a pot containing 2 L of water. Sampling for aflatoxin fate was made 15 min, 30 min, 45 min, 60 min, 90 min and 2 h after onset of the first vapors.

Analysis of aflatoxin content

Samples of peanut and peanut products from the market are stored in the chill room at 4 – 6 °C prior to analysis of aflatoxin. From the market sample, an aliquot of 100 g peanut kernels was ground in a Waring Blender at high speed prior to extraction of aflatoxin. For the cooking tests however, two samples of 50 g each were taken during the cooking process to determine the aflatoxin content on one hand and on the other hand the water content of the sample.

Aflatoxin extraction was performed on 25 g flour added with 100ml 80% methanol and 5 g NaCl in a glass jar of a Waring Blender and mixed at high speed for 5min. After filtration through a No 4 filter paper, the liquid phase was submitted to a second filtration with glass fiber filter paper. The final extract was submitted to aflatoxin quantification.

AflaTest® Immunoaffinity (VICAM) chromatography was performed according to the manufacturer's instructions. Briefly, 5 mL of diluted sample extract (2:3, extract:water) was passed through an immunoaffinity column at one drop per second. The column was washed with 10 mL of water by passing through the column at two drops per second. Aflatoxin was eluted from the column by passing through 1mL of methanol

Table 1 aflatoxin content of peanut products from different markets of Thies (N=20 $p \leq 0.05$)

Peanut product	Positive samples (%)	Samples containing more than 10 ppb * (%)	Min - Max (ppb)	Average
First choice peanut	80	0	0 - 6.50	1.25± 0.31
Second choice peanut	100	50	0.55 - 15.33	4.43 ±2.13
Peanut flour	100	0	0.30 - 6.63	2.17± 0.22
Peanut butter	100	0	0.83 - 3.16	1.83± 0.13

10 ppb is the maximum total aflatoxin content in Food tolerated by the European Union

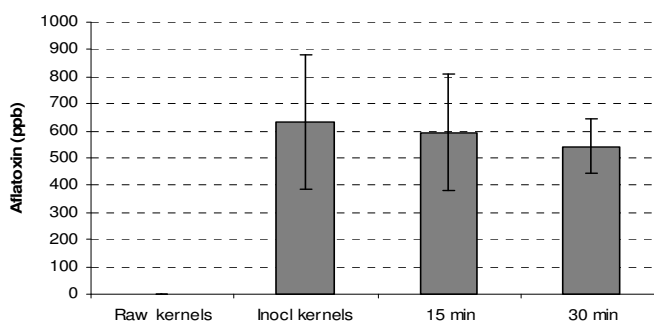


Figure 1 Influence of steaming on aflatoxin content of flour made from first choice peanut inoculated with *A. flavus* M12-12 (N = 9, $p \leq 0.05$); (Raw kernels = non inoculated first choice peanut, Inocl kernels = non processed inoculated first choice peanut).

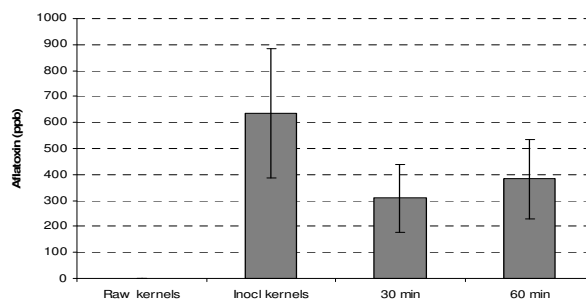


Figure 2. Influence of boiling on aflatoxin content of flour made from peanut inoculated with *A. flavus* M12-12 (N = 9, $p \leq 0.05$); (Raw kernels = non inoculated first choice peanut, Inocl kernels = non processed inoculated first choice peanut).

at one drop per second. Into the eluent was added 1 mL of developer solution provided by the manufacturer, prior to reading using Vicam AflaTest® Series 4-

fluorimeter. For the cooking tests, the aflatoxin readings were then adjusted to sample dry weight.

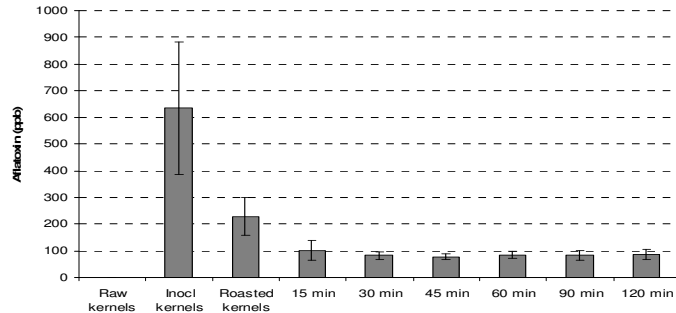


Figure 3 Influence of boiling on aflatoxin content of butter made from peanut inoculated with *A. flavus* M12-12 (at N = 9, $p \leq 0.05$); (Raw kernels = non inoculated first choice peanut, Inocul kernels = non processed inoculated first choice peanut).

Data analysis

Data were summarised and analysed using SAS (version 9.1, SAS Institute, Cary, NC). The means were separated using analysis of variance (ANOVA) and Tukey test (95% confidence interval) to determine whether there were significant differences between the samples.

RESULTS

Aflatoxin content of peanut products from the markets

Samples of first-choice peanut kernels from the markets in Thies had a low aflatoxin level (1.25 ppb) (Table 1). Aflatoxin content varied between 0 and 6.50 ppb. About 80% out of the 20 samples tested were found to contain aflatoxins, but none reached the threshold value for commercial goods of 10 ppb for total aflatoxins, set by the European Union. The "second choice peanut" had the highest aflatoxin content (4.43 ppb) with values ranging from 0.55 to 15.33 ppb. They all tested positive to aflatoxin, with 50% samples above the 10 ppb mark.

All peanut flour ("Noflaye") samples tested positive with values ranging from 0.30 to 6.63 ppb, with a mean value of 2.20 ppb. Aflatoxin values for peanut butter ("Degue") samples were even lower with 1.83 ppb in average. The samples tested positive at 100%, with values varying between 0.83 and 3.16 ppb. The highest mean values were recorded for sechoice peanut and peanut flour with significant differences with regard to first-choice peanut and peanut butter. The first-choice peanuts had the lowest aflatoxin content.

Influence of steam cooking of flour on aflatoxin content

Inoculation of first choice peanut with *A. flavus* M12-12 and incubation at room temperature for five days raised

the aflatoxin content of the peanut from 0.28 to 635 ppb (Figure1).

Steam cooking of flour made from inoculated peanut led to a decrease of aflatoxin content within 15 min (from 635 ppb to 593 ppb). Longer cooking resulted in further decrease of aflatoxin level (540 ppb). The difference to the inoculated and non processed peanut was still, however, not significant.

Influence of boiling on aflatoxin fate of peanut flour

When the flour made from the inoculated peanut was submitted to boiling, aflatoxin content was decreased in average from 635 ppb to 322 ppb after 30 min (Figure 2). A longer cooking time did not show further improvement. Aflatoxin content of the uncooked inoculated control sample was not significantly different to that of the cooked peanut.

Influence of water cooking on the aflatoxin fate of peanut butter

Roasting peanuts at 140 °C for 1h, drastically decreased the aflatoxin content of the peanut, from 635 to 229 ppb (Figure 3). The differences between aflatoxin value of roasted peanut and the inoculated control was highly significant ($p < 0.01$). Water cooking of peanut butter further decreased the aflatoxin content. Roasting alone reduced aflatoxin content by about 64%. Boiling the peanut butter enhanced aflatoxin reduction that reached 84.25%, decreasing from 635 ppb down to 118 ppb after 15 min boiling. Further cooking did not show improvement.

DISCUSSION

This study provides the first data related to the effect of different cooking methods commonly used in Senegal on peanut aflatoxin fate.

Aflatoxin content of market samples depended on the peanut quality as well as the type of product. First-

quality peanuts had low aflatoxin content, therefore confirming the efficacy of sorting as a method to manage aflatoxin exposure (Galvez et al., 2003). In contrast, the second-choice peanuts, in addition to testing positive to aflatoxins for all samples, had aflatoxin levels above 10 ppb in 50% of the samples. Sorting into first and second-choice peanuts have been reported to concentrate the maximum aflatoxin risk into the second-choice fraction (Whitaker et al., 1998). Aflatoxin content of peanut butter (“degue”) (1.83 ppb) and flour (“Noflaye”) (2.17 ppb) were higher than that of first choice peanut, suggesting that these products were made from second-choice peanuts. This is an indication of a frequent use of second-choice peanut (with higher aflatoxin levels) for the preparation of peanut-derived products. In fact, it is very usual that after sorting, good quality peanut are sold because of their higher price; the rest is often processed to make peanut flour or butter for local consumption (Diouf et al. 2005). This peanut fraction is the most commonly eaten in rural areas where poverty is most widespread. The significantly lower aflatoxin content of peanut butter with regard to second choice peanut and peanut flour (from unroasted peanut) indicates a reduction of toxin content through roasting as demonstrated by Ogunsanwo et al. (2004 and 2005).

The first-choice peanuts had low aflatoxin content. Inoculation with *A. flavus* M12-12 caused a high increase of aflatoxin content within 5 days. Boiling was able to induce a 52% reduction of aflatoxin content of peanut flour, in the same range as Stoloff *et al.*, (1981) who obtained 53% aflatoxin reduction through boiling maize flour. Boiling aflatoxin B1 contaminated pasta (macaroni and noodles) led to a reduction by about 29% (Lopez-Garcia et al., 1999). The reduction obtained through boiling in the present study was, however, not statistically significant. This poses a crucial problem with regard to dietary habits of Senegalese people in the Peanut Basin in particular. Intake of dishes like “sombi guerte” and “mbaxal”, likely made from second-choice peanut that concentrates the aflatoxin contamination risk (Whitaker et al., 1998), may be conducive to frequent exposure to high aflatoxin levels. In rural socio-economic conditions Fingani et al. (2004) found aflatoxin content from 12 to 329 ppb in peanut from retail sale in Botswana. Moreover aflatoxin-albumin adducts were found twice as high in blood samples from Gambian rural residents as from people from peri-urban localities (Wild et al., 2000). High aflatoxin-albumin adducts were also found in blood of Ghanaians and Guineans as a consequence of dietary exposure to the toxin (Jiang et al., 2005; Turner et al., 2005). This may be an indication of high exposure risk of rural residents from the Senegalese Peanut Basin where groundnut is a staple food eaten on a daily basis.

Steaming of aflatoxin-contaminated peanut flour brought no significant reduction. This is indicative to aflatoxin intake through the use of non sorted peanut for meals like “mbaxal” where the peanut flour is added to the almost-cooked rice, millet or sorghum to be

steam cooked for about 15 minutes. The same conclusions could be drawn for peanut based dishes where boiling or steaming are the only cooking methods used. The analysis of the effect of different cooking methods of maize in Benin showed the small effect of boiling in reducing aflatoxin content (Fandohan et al., 2004). Moderate reduction of aflatoxin content of peanut and maize through traditional cooking (boiling) was reported by Njapau et al. (1997) in Zambia. Park et al. (2005) found cooking to reduce rice aflatoxin content by 34%. In the present study however 52% reduction was observed but the value was still not significantly different with regard to the inoculated and uncooked peanut. The temperature during these two cooking methods did not exceed 80-85 °C as also reported by Miren et al. (2006).

Roasting brought 64% reduction of aflatoxin level. This process is used for the preparation of “mafe”, a popular dish in West Africa but also for Senegalese dishes like “dakhin” and “ngalakh”. Roasting to get the appropriate peanut paste required an exposure to high temperatures (140°C) for 1 h. Roasting at minimum temperatures of 140°C for 40min was reported to reduce drastically aflatoxins in peanut (Ogunsanwo et al., 2004; Ogunsanwo et al., 2005; Njapau et al., 1997). The decrease of 64% aflatoxin content through roasting at 140°C for 60 is consistent with observations of Conway et al. (1978) who found that grilling corn (between 145 and 165°C) results in a reduction of 40 to 80% aflatoxin content. According to Marth and Doyle (1979), roasting contaminated peanut could be a very effective strategy to reduce aflatoxin. Similar conclusions indicating that roasting pistachio may lead to a significant reduction of contamination levels ranging from 17 to 63% were drawn by Yazdanpanah et al. (2005). Moreover, high temperature in food processing like baking (200°C) leads to a reduction of aflatoxin content by 90% (Lee, 2006).

When peanut butter from the roasted peanut was submitted to boiling, the reduction of aflatoxin content became more important (84%). It could therefore be anticipated that eating “mafe” and “dakhin” that include further cooking of peanut butter could be less risky than eating “ngalakh” for which the peanut butter is not processed.

“Mbaxal” and “sombi guerte”, prepared by only boiling unroasted peanut flour may be high-risk dishes when aflatoxin-contaminated peanut are used. Castells et al. (2008) suggested that neither cooking nor roasting had a significant effect on aflatoxins in flaking maize grits, despite steps in the process involving pressure cooking at 110 °C and roasting at 300 °C. However, Ogunsanwo et al. (2005) showed that aflatoxin reduction through roasting depends upon both temperature and time. Combining different methods along the peanut production and processing chain could be more effective against aflatoxin, as roasting alone did not provide sufficient control of aflatoxins in some cases in particular when temperature and time combination during roasting are not adequate (Bankole et al., 2004).

A good mitigation strategy to efficiently control aflatoxin risk in food could be achieved through the use of good quality peanut in combination with different cooking methods during preparation. Dry roasting at appropriate temperatures and time could be very efficient to reduce aflatoxin risk.

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