

Oil Contents and Aflatoxin Levels in Peanuts Varieties Produced in Busia and Kisii Central Districts, Kenya

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Abstract

Busia and Kisii Central districts have repeatedly reported high levels of aflatoxins in foods. The objectives of the study were to determine oil contents and the relationship between oil contents and the levels of total aflatoxin in peanuts varieties produced in the two districts. Three samples of each of the four (4) varieties of peanuts obtained from Busia; Valencia red, Uganda local, Homa Bay local and local red and 3 varieties from Kisii Central; Valencia red, Uganda local and Homa Bay local were analyzed and the average oil contents of the samples in each variety determined. The oil contents of peanuts were determined by a standard Soxtec extraction method and total aflatoxins were analyzed using high performance liquid chromatography (HPLC) technique. Peanuts from Busia district had significantly higher oil contents compared to those from Kisii Central ($t=3.22$, $df=6$, $P=0.012$). Peanuts of Valencia red variety from both Busia and Kisii Central had higher oil content (mean 46.9), than other varieties. In addition, Valencia red from Busia district had slightly higher oil content (47.2%) than those of the same variety from Kisii Central (46.6%). However, the difference was not significant ($t=1.08$, $df=6$, $P=0.394$). Overall, oil content in peanuts decreased with an increase in aflatoxin levels ($r=-0.496$, $P=0.031$) except in peanuts of Uganda local red variety from Kisii Central. Growing of improved varieties of peanuts such as Valencia red which was least contaminated with aflatoxin and had higher oil content should be encouraged in Kenya.

Keywords: Peanuts; Oil contents; Levels of aflatoxins

Introduction

Aflatoxins are mycotoxin produced by *Aspergillus* species which grows in peanuts. Peanuts and peanuts oils are widely consumed in Kenya and peanut contamination with aflatoxins pose health risks to humans [1]. A large number of compounds originated from plants including essential oils from foods and microorganisms have been proven as strong inhibitors of aflatoxin (AF) biosynthesis [2]. Inhibition of growth and aflatoxin production by *A. flavus* and *A. parasiticus* by spice oils and their active components has been reported [2].

Studies towards identification of the target sites of these inhibitors have shown that they may act via interfering with the signal transduction regulatory networks involved in AF gene expression, blocking activities of AF biosynthetic cytosolic enzymes, down-regulating fungal genes of the oxidative stress defense system that combats metabolic and environmental stressors. The oils also inhibit fungal pathogenesis factors, disrupting mitochondrial respiration, a critical process that provides acetyl-CoA for AF biosynthesis and they are also associated with morphological alterations in the mycelium, such as vacuolation of cytoplasm and attenuation of cell wall [3]. This suggests that the integrity of the cell barriers particularly that of the cell wall, may be crucial in the regulation of aflatoxin production and excretion [3]. There are no studies documenting the effect of peanut oil on growth of *A. flavus* and aflatoxin production in Kenya whereas such knowledge would be important in selection of peanut varieties for

farming that are resistant to *Aspergillus* fungi that produces aflatoxin. The current study therefore evaluated the effect of peanut oils on growth of *Aspergillus* species and aflatoxin production in peanuts collected from Busia and Kisii Central districts.

Materials and Methods

Study areas

The study was conducted in two districts in western Kenya namely Busia and Kisii Central which represented Busia and Kisii counties respectively are the main peanut producing areas in Kenya [4] and have a high demand for peanuts and their products.

Study design

A cross-sectional study was adopted among the peanuts farmers in Busia and Kisii Central districts.

Study population

The study comprised of peanuts farmers in Busia and Kisii Central districts. The number of farmers growing groundnuts varied between different agro-ecological zones (divisions) within each district. The agro-ecological zones were determined based on altitude, mean annual rainfall, temperature and probability of successful growing of peanuts

in that zone. The peanuts production statistics of farmers were obtained from the division's agricultural offices [4].

Sampling technique

Peanuts farmers' households were sampled from the agro-ecological zones (divisions). In Busia, farmers in the leading groundnut producing divisions; Butula, Matayos, Funyula and Budalangi were purposively selected. The other division in Busia (Township) had only a small area under groundnuts cultivation was not included in the study. In Kisii, farmers in the four leading peanut producing divisions; Keumbu, Masimba, Suneka and Mosochi were also purposively sampled. The other divisions in the district (Kisii Town and Marani) were excluded from the study due to small areas under groundnuts production. Within the divisions selected in both Busia and Kisii Central districts, peanuts farmers households were randomly selected by staggering every fourth household within the division administrative boundary, the starting point being the fourth household from the division's agricultural office in the particular divisions. The sampling interval was obtained based on the approximate peanuts farmers' population of 408 in the study areas [5] divided by the sample size (102).

Study approval, ethical consideration and informed consent

Ethical approval was obtained from Kenyatta University Ethics Review Committee. The study objectives were explained to the peanuts farmers and they were allowed to ask questions. After giving consent, the participants signed a consent form and peanuts samples were collected. The participants were assured that information obtained would be treated with confidentiality.

Laboratory Procedure

Sample collection

A total of 204 peanut samples of different varieties were collected in the two districts using the procedure of Whitaker [6]. Peanuts stored in sacks or boxes were sampled from different parts using a closed spear driven through the top and sides of each sack or box to obtain a total of 0.5 kg of sample. Each of the 0.5 kg samples of unsorted peanuts were put in clean polyethylene bags, sealed, labeled and put in cool boxes. They were then transported taken to Bora Limited Laboratory, Nairobi and University of Nairobi, Department of Food Science, Nutrition and Technology and stored at 4°C. In Busia district, the 102 peanuts samples were of four different varieties; Valencia red, Uganda local, Homa Bay local and Local red. The 102 peanuts samples from Kisii Central district were of three different varieties; Valencia red, Uganda local and Homa Bay local. In both districts, Valencia red variety had the most number of the samples, 59 and 89 from Busia and Kisii Central districts respectively which were significantly different from the other varieties ($\chi^2=12.00$, $df=9$, $P=0.02$). There were more samples of Uganda local red (21) and Homa Bay local (20) varieties from Busia district compared to those from Kisii Central district. Local red variety had only 2 samples in Busia and none in Kisii Central (Table 1).

Peanut Variety	Number of peanut samples collected		
	Busia	Kisii Central	Total
Valencia red	59	89	148
Uganda local red	21	5	26
Homa Bay local	20	8	28
Local red	2	0	2
Total	102	102	204

Table 1: Peanut varieties from Busia and Kisii Central districts.

Aflatoxin analysis

Each homogenized grounded peanut sample was triturated and analyzed to determine the types and levels of aflatoxin contaminants.

Extraction of aflatoxins from peanuts powder: Fifty grams from each homogenized peanuts sample was triturated in a blender in 50 ml of 70% methanol (70 ml absolute methanol mixed in 30 ml distilled water) containing 0.5% potassium chloride until thoroughly homogenized. The extract was transferred to a conical flask and centrifuged at 300 rpm for 30 minutes to remove any particulates. The extract was filtered through Whatman No.4 filter paper and diluted 1:10 in phosphate buffered saline containing 500 µl/L Tween-20. Analysis for aflatoxins was done using High Performance Liquid Chromatography (HPLC).

Identification and quantification of aflatoxins: High Performance Liquid Chromatography was used to confirm identity of the aflatoxin and quantified using the method of Bragulat et al. [7]. HPLC system consisted of a Hewlett Packard 1100 pump (Palo Alto, CA, USA) connected to an HP 1046A programmable fluorescence detector and quantification was done with an HP workstation. Twenty microliters (20 µl) of each extract was applied to HPLC and chromatographic separation was performed on a stainless steel C18 reversed phase analytical column (150×4.6 mm, 5 µm particle size) preceded by a C18 pre-column (Ultraprep 104 mm). Water: methanol: acetonitrile (4:1:1, v/v/v) was used as the mobile phase at a flow rate of 1.5 mL/min. Fluorescence of aflatoxin derivatives was recorded at excitation and emission wavelengths of 360 nm and 440 nm respectively. Aflatoxins were quantified based on HPLC fluorometric response compared with aflatoxin standards (Sigma chemical, St. Louis, MO, USA). The limit of detection was 0.005 µg/kg for AFB1 and AFG1, and 0.02 µg/kg for AFB2 and AFG2.

Determination of oil contents of peanuts

One sample from each of the four (4) varieties of peanuts collected from Busia and the 3 varieties of peanuts (Valencia red, Uganda local and Homa Bay local) collected from Kisii Central districts were analyzed for oil contents. The oil contents of peanuts were determined by a standard Soxhlet extraction method using the procedure of Sundaram et al. [8]. Briefly, about 5 grams of the homogenized ground peanut sample was accurately weighed into an extraction thimble. The sample was then covered with cotton wool and the thimble was placed into the Soxhlet extractor. A flat bottomed flask with 200 ml of

petroleum was placed on a heating mantle and connected to the Soxtec extractor and the extraction started. The extraction of oil continued for about 8 hours. After 8 hours, the solvent was evaporated in a rotary evaporator. The residue was then dried in an air oven at 105°C for 1 hour. The crude oil content of the sample was then calculated as a percentage by dividing the weight of the crude oil by the weight of the sample (5 grams), multiplied by 100%.

Data analysis

The distribution of samples in the levels of aflatoxins was determined using Mann-Whitney U test. The difference in oil contents of the different peanuts varieties from the two districts and the relationship between the oil content and the aflatoxin levels in the samples were determined using correlation test. All the statistical analysis was done using MINITAB and SPSS version 19.0 computer package.

Results

Oil contents of the different varieties of peanuts

Using a paired T-test, peanuts from Busia district had significantly higher oil contents compared to those from Kisii Central ($t=3.22$, $df=6$, $P=0.012$). Peanuts of Valencia red variety from both Busia and Kisii Central had higher oil content (mean 46.9), compared to other varieties (Table 2). In addition, Valencia red variety peanuts from Busia district had slightly higher oil content (47.2%) than peanuts of the same variety from Kisii Central which had 46.6% although the difference was not significant ($t=1.08$, $df=6$, $P=0.394$). Peanuts of Local red variety from Busia district had the lowest oil content (42.7%) compared to other varieties from the same district ($t=2.28$, $df=6$, $P=0.026$). Homa Bay local variety peanuts from Kisii Central had the lowest oil content (40.6%) among all the other peanuts varieties analyzed (Table 2).

Peanuts variety	Average (%)		Overall Mean (%)
	Busia	Kisii Central	
Valencia red	47.2	46.6	46.9
Uganda local red	46.7	45.7	46.2
Homa Bay local	43.2	40.6	41.9
Local red	42.7	-	42.7

Table 2: Oil contents of different varieties of peanuts from Busia and Kisii Central districts.

Relationship between oil contents and the levels of total aflatoxin in peanuts

Oil contents of peanuts from Busia and Kisii Central districts were compared with the total aflatoxins of the same samples. All the four peanuts varieties from Busia had lower levels of total aflatoxins except the Local red variety which had the highest total aflatoxins levels at 267

µg/kg but with the lowest oil content average of 42.7% among peanuts varieties from Busia (Table 3). Peanuts varieties from Kisii Central had high levels of total aflatoxin but low oil contents. Peanuts of Uganda local red from Kisii Central had the highest total aflatoxin levels of 405 µg/kg and average oil content of 45.7%. Overall, there was an increase in total aflatoxin levels with decreasing oils contents ($r=-0.496$, $P=0.031$) except in peanuts of Uganda local red variety from Kisii Central district (Table 3).

District	Peanut variety	Average oil content (%)	Total Aflatoxin level in the sample (µg/kg)
Busia	Valencia red	47.2	2.3
	Uganda local red	46.7	2.4
	Homa Bay local	43.2	2.8
	Local red	42.7	267
Kisii Central	Valencia red	46.6	93
	Uganda local	45.7	405
	Homa Bay local	40.6	101.5

Table 3: Oil contents in relation to total aflatoxins in peanuts analyzed.

Plotting oil content of peanut from Busia against Aflatoxin levels showed a reduction of oil content with increase in Aflatoxin levels ($r=-0.0114$, $P=0.051$) and those from Kisii Central also showed decrease of oil contents with an increase in aflatoxin levels ($r=-0.0064$, $P=0.045$) except Homa Bay local variety as illustrated in Figures 1 and 2 respectively.

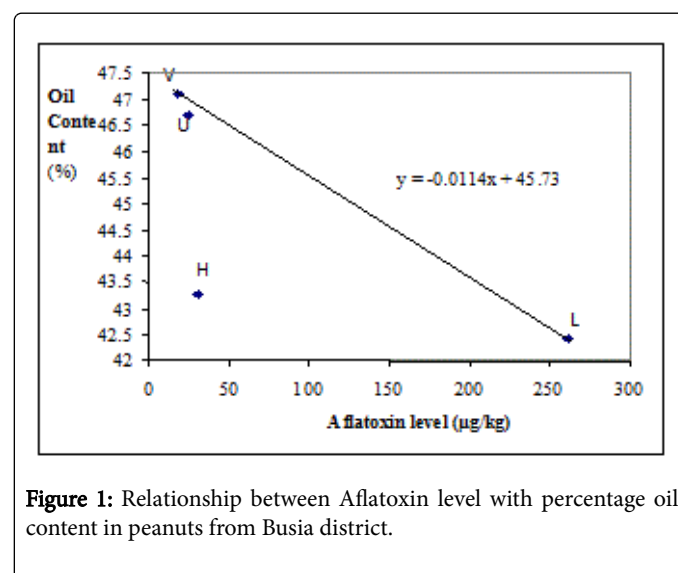


Figure 1: Relationship between Aflatoxin level with percentage oil content in peanuts from Busia district.

V=Valencia red; U=Uganda local red; H=Homa Bay local; L=Local red.

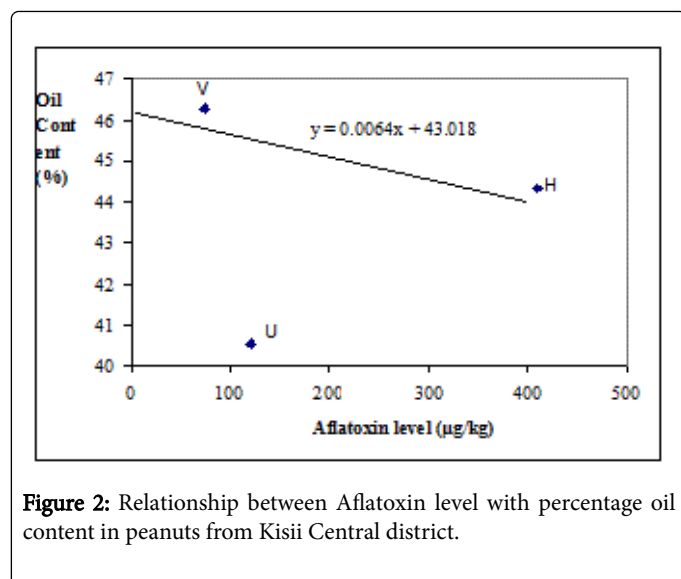


Figure 2: Relationship between Aflatoxin level with percentage oil content in peanuts from Kisii Central district.

V=Valencia red; U=Uganda local red; H=Homa Bay local.

Discussion

Peanuts varieties from Busia and Kisii Central districts

In both districts, peanuts of Valencia red variety had the highest number of samples, 59 and 89 from Busia and Kisii Central districts respectively which were significantly different from the other varieties. This could have been attributed by the fact that Valencia red variety is the most common improved variety of peanuts planted by farmers in the western region of Kenya [9] compared to other varieties in the study areas. The variety tends to be preferred due to higher yield and resistance to diseases which may also reduce their susceptibility to infection by *Aspergillus* species compared to the local varieties [9]. The result of this study is in line with that of Mutegi et al. [9] who documented a higher number of peanuts of Valencia red variety than the other varieties in the study areas.

In this study, there were few samples of peanuts of local varieties; Uganda local red (26), Homa Bay local (28) and Local red (2) from the two study districts. It is possible that peanuts farmers avoid planting these varieties because they have low yields and are less resistant to diseases [9]. Moreover, local varieties of peanuts such as Uganda local red, Homa Bay local and Local red, have been reported to be more susceptible to rosette virus and mould than improved varieties [4], and a positive correlation between the diseases and aflatoxin contamination of peanuts have been documented in other studies [11]. These findings are consistent with those reported in previous studies by Mutegi et al. [12].

Oil contents of the different varieties of peanuts from Busia and Kisii Central districts

Essential oils from foods and microorganisms have been proven as strong inhibitors of aflatoxin biosynthesis [1]. Inhibition of growth and aflatoxin production by *Aspergillus flavus* and *A. parasiticus* by spice oils has been reported [13]. In the current study, peanuts from Busia district had significantly higher oil contents compared to those from Kisii Central district ($t=3.22$, $df=6$, $P=0.012$). This could be because the climate in Busia is hot and dry which enhances formation of oils

compared to Kisii Central which is wet [9]. Being the first study to be conducted in Kenya to determine oil contents in peanuts, there is no data to be compared with the results of the current study. However, the result is in line with a previous study on peanuts done in Turkey [14].

Peanuts of Valencia red variety from both Busia and Kisii Central had higher oil content (mean 46.9), compared to other varieties analyzed in the study. This could probably be due to the fact that Valencia red is an improved peanut variety which takes shorter time to mature especially in dry climate and therefore very fast in the formation of oil compared to the other varieties analyzed. However, Local red variety peanuts from Busia and Homa Bay local from Kisii Central had the lowest oil content at 42.7% and 40.6% respectively. This could probably attribute to the longer time the local varieties take to mature compared to the improved varieties and therefore low formation of oil [13].

Relationship between oil contents and the levels of total aflatoxin in the peanut samples

Oil contents of peanuts from Busia and Kisii Central districts were compared with the total aflatoxins of the same samples. Overall, there was increased total aflatoxin levels with decreasing oils contents ($r=-0.496$, $P=0.031$) except for Uganda local red variety from Kisii Central district. This is because low oil content in peanuts partially blocks activities of aflatoxin biosynthetic cytosolic enzymes hence increasing production of Aflatoxin production and vice versa. In addition, oils are known to disrupt fungal mitochondrial respiration that provides acetyl CoA for Aflatoxin biosynthesis and therefore the higher the oil content, the lower the Aflatoxin production by *Aspergillus* species [13]. The results are consistent with a report of a study done by Reddy et al. [13] who reported an increased inhibition of growth and production of aflatoxin by *Aspergillus flavus* and *A. parasiticus* by increased concentration of spice oils and their active components. Therefore, the higher the oils contents, the higher the inhibition of growth and production of aflatoxins by *Aspergillus* species.

As stated earlier, the only exception in the study was observed in peanuts of Uganda local red variety from Kisii Central district which had the highest level of total aflatoxins (405 µg/kg) compared with other varieties in the study. This variety also had high average oil content (45.7%). The result could be explained by other factors other than oil content such as higher susceptibility of local peanuts varieties to crop diseases and pests that result in plant stress predisposing them to aflatoxin contamination [15]. Local peanuts varieties such as local red, Homa Bay local and Uganda local red have been reported to be susceptible to stem rot and mould [4] and a positive correlations between these diseases and aflatoxin contamination of peanuts have been reported [16]. The result is in line with the study of Mutegi et al. [12] who reported high aflatoxin levels in peanuts of local varieties compared to the improved ones.

Samples of all peanuts varieties from Busia had lower levels of total aflatoxins with an increase of oil content except the Local red variety. This variety had significantly higher total aflatoxins levels at 267 µg/kg and 42.7% average oil content. The result could probably be contributed by factors other than oil contents such as susceptibility to crop diseases and pests by local peanuts varieties that result in plant stress predisposing peanuts to growth and aflatoxin contamination by *Aspergillus* species. These results concur with the study of Hell et al. [10], who reported a positive correlation between the growing of local

varieties and increased Aflatoxin levels in peanuts in Benin. Overall, peanuts from Busia district had high levels of oil contents and low level of total aflatoxins compared to those from Kisii Central which had slightly lower oil content and significantly higher total aflatoxins levels.

Conclusion

Valencia red peanut variety is mainly grown in the two study areas. Oil content in different varieties of peanuts from Busia district was slightly higher than those from Kisii Central. However, the oil contents in peanuts decreased with an increase in aflatoxin levels ($r=-0.496$, $P=0.031$) except peanuts of Uganda local red variety from Kisii Central. The study recommends further investigations on the link between oil contents and aflatoxins production by *Aspergillus* species as this was the first study to be carried out in Kenya.

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