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ORIGINAL ARTICLE

Effects of packaging and pre-storage treatments on aflatoxin production in peanut storage under controlled conditions

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Abstract This study reports on aflatoxin production and peanut (Bailey's variety) quality, for four peanut pre-storage treatments; [(Raw clean (Raw-Cl), Raw inoculated with *Aspergillus flavus* NRRL 3357 (Raw-Inf), inoculated partially roasted but not-blanched (PRN-blanch); and inoculated partially roasted, blanched with discolored nuts sorted out (PR-blanched)]. All four treated samples were packaged in four different packaging systems [polypropylene woven sacks (PS), hermetic packs (HP), hermetic packs with oxygen absorbers (HPO), and vacuumed hermetic packs (HPV)] and stored under controlled conditions at a temperature of 30 ± 1 °C and water activity of 0.85 ± 0.02, for 14 weeks. Raw-Inf samples in PS had a higher fungal growth with a mean value of 8.01×10^4 CFU/g, compared to the mean values of samples in hermetic packs: 1.07×10^3 CFU/g for HP, 14.55 CFU/g for HPO, and 57.82 CFU/g for HPV. Similarly, the hermetic bags were able to reduce aflatoxin level of the Raw-Inf samples by 50.6% (HP), 63.0% (HPV), and 66.8% (HPO). Partial roasting and blanching in PS also reduced aflatoxin level by about 74.6%. Quality maintenance was the best for peanuts in HPO, recording peroxide value (PV) of 10.16 meq/kg and *p*-Anisidine (*p*-Av) of 3.95 meq/kg compared to samples in polypropylene woven sacks which had PV of 19.25 meq/kg and *p*-Av of 6.48 meq/kg. These results indicate that using zero-oxygen hermetic packaging, instead of the conventional polypropylene woven sacks, helped to suppress aflatoxin production and quality deterioration. Also, partially roasted, blanched and sorted peanuts showed a potential for reducing aflatoxin presence during storage.

Keywords Peanuts · *Aspergillus flavus* · Aflatoxin · Lipid oxidation · Hermetic storage

Introduction

Aflatoxin contamination of peanuts and peanut-based products is of great concern because it is associated with carcinogenicity and toxicity in both humans and animals (Magnussen and Parsi 2013). Magnussen and Parsi (2013) continue to explain that chronic aflatoxicosis can result in prolonged pathologic changes, including cancer and immunosuppression and acute aflatoxicosis can also result in death. This is a great concern in Sub-Saharan Africa because peanuts are extensively used in preparing all kinds of dishes, mixed with baby's food as a protein source, or used as a bread-spread. The level of aflatoxin contamination also creates a trade barrier and can result in great economic losses to exporters and the region at large.

Peanuts and other tree nuts are affected by aflatoxin, the secondary metabolite of *Aspergillus*, and are compounded during storage due to favorable environmental conditions and inadequate storage structures. Ghana and most African countries have average temperatures and humidity that favor the growth *Aspergillus* fungi and

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aflatoxin production. Kaaya and Kyamuhangire (2006) reported that most food contamination occurs during post-harvest storage and aflatoxin contamination of food has been shown to increase with storage period. In market places, mostly shelled peanuts are packaged in jute or polypropylene woven sacks, sisal (straw woven) bags, but the packaging material used were also found to influence aflatoxin levels in the stored peanuts (Mutegi et al. 2013). These packaging materials are not airtight, and there is evidence that these methods of storage facilitate fungal contamination and aflatoxin development (Hell et al. 2000).

Storage structures and conditions can greatly influence aflatoxin production in peanuts, but there are some measures that can be used to control aflatoxin levels. Physical, chemical and biological control measures are available for controlling aflatoxin (Jalili et al. 2010). Some of the controlling methods have shown positive results (e.g., Basaran and Akhan 2010; Kumar et al. 2010). Unfortunately, some of these methods are not suitable for application to food, and can make the resultant products unwholesome for human consumption (Akbas and Ozdemir 2006). For example, ammonia fumigation has been shown to decrease aflatoxin levels, but was not been approved by the Food and Drug Administration (FDA) due to toxicity of its reaction products (Park et al. 1987).

Consequently, methods that are safer, economically feasible, practical and, most importantly, implementable in the African market, need to be developed. Peanut is mostly consumed in the roasted form in most parts of the world (Ogunsanwo et al. 2004). Some studies have shown that roasting helps to reduce the aflatoxin level (Ogunsanwo et al. 2004; Yazdanpanah et al. 2005). Also, several studies have reported *Aspergillus* growth and aflatoxin production on peanuts under controlled conditions with hermetic packaging (Ellis et al. 1994; Vaamonde et al. 2006; Garcia et al. 2011; Navarro et al. 2012). However, studies on the use of hermetic storage for preventing aflatoxin in longer-term storage are limited.

Considering all of the above, it is clear that there is the need for a practical, economic, and easily applicable technique to store peanuts under controlled conditions to manage the aflatoxin contamination and aflatoxigenic fungi growth; and to reduce peanut quality deteriorations in developing countries. This experimental study was conducted to determine the optimum condition to limit *Aspergillus* growth, aflatoxin production and lipid oxidation in peanuts. The effect of four different pre-storage treatments of peanut and four different packaging systems were investigated.

Materials and methods

Experimental design

A 4 × 4 factorial design was used (i.e., four peanut processing methods and four types of packaging systems). The four pre-storage treatments were: (1) Raw clean peanuts (Raw-Cl); (2) Raw peanuts inoculated with *A. flavus* NRRL 3357 (Raw-Inf); (3) partially roasted, not blanched peanuts inoculated with *A. flavus* NRRL 3357 (PRN-blanch); and, (4) partially roasted, blanched peanuts inoculated with *A. flavus* NRRL 3357 (PR-blanched). PR-blanched samples were sorted to remove discolored nuts. The four packaging systems were: (1) polypropylene woven sacks (PS); (2) hermetic packs (HP); hermetic packs with oxygen absorbers (HPO); and, (3) vacuumed hermetic packs (HPV). The 16 treatment combinations were each replicated 15 times for a total of 240 experimental units. Three experimental units of each treatment were randomly selected, terminated, and analyzed at 0, 2, 6, 10, and 14 weeks.

Aspergillus spp. spore suspension preparation

Aspergillus flavus NRRL 3357 (aflatoxigenic fungi) from stock cultures was added to potato dextrose agar and incubated at room temperature for 5 days to enable significant sporulation to take place. After incubation, 10–15 ml of sterile distilled water was added to each plate. A sterile plastic inoculation loop was used to loosen the conidia from the PDA plates. The suspension was then filtered through sterile cheesecloth into a sterile 50 ml Falcon tube. Spores were enumerated using a hemocytometer (Bright-line™, Sigma-Aldrich, St. Louis, MO, USA) and a microscope (Leica DME, Meyer Instruments, Inc., Houston, TX, USA), and appropriate dilutions were made before enumeration.

Peanuts rehydration and inoculation

Virginia type peanut variety (Bailey's) was obtained from Tidewater Agricultural Research and Extension Center. Shelled peanuts were visually inspected and defective peanuts were discarded. The remaining peanuts were partially sterilized under UV light in a biosafety cabinet for 30 min and then the moisture content was adjusted to desired value of 10% by adding sterile distilled water to the substrate in sterile bottles. The bottles were cooled down to 4 °C and stored at 4 °C for 48 h with periodic shaking as described by Garcia et al. (2011). The amount of water necessary to reach the 10% moisture content was determined by using the following equation:

$$\frac{(MC_{final} - MC_{initial}) \times W}{100 - MC_{final}} = H_2O \quad (1)$$

where $MC_{initial}$ is moisture content before hydration, MC_{final} is final or desired moisture content (%), W is weight of peanuts to be hydrated (g), and H_2O is amount of water needed for the hydration (ml).

Moisture content was confirmed with an infrared moisture meter (Ohaus MB 200, H & C Weighing Systems, and Columbia, MD, USA) and the corresponding water activity value was also confirmed with water activity meter (Decagon devices Inc, WA, USA). For inoculation, each kernel was infected by pipetting 10 µl spore suspensions (10^5–10^6 CFU/ml) of *A. flavus* NRRL 3357 on the surface. Infected peanuts were then incubated at a temperature of 30 ± 1 °C and relative humidity of 85 ± 3% for 48 h before various treatments were applied and the peanuts were packaged. Twenty micro liters (20 µl) of spore suspension was applied to each peanut surface and left in the incubator for 2 days.

Preparation of peanut treatments and packaging

After manually removing discolored, moldy and defective peanuts, those used were divided into four batches. The first batch of peanuts did not receive any treatments and was labeled as Raw-Cl. This batch was then packed into the four different packaging systems and sealed. The second batch, labeled as Raw-Inf, was prepared by adding 2-g of previously inoculated peanuts to 98-g of clean peanuts. The third and the fourth batches were partially roasted. These samples were roasted in sub batches of 1 kg for 10 min at 145 °C. Half of this roasted lot was blanched (peeling off the testa), and the remaining not blanched. The inoculated samples were partially roasted separately under the same conditions and 2-g of them were added to each package (PRN-blanch). Inoculated and partially roasted samples were blanched and discolored nuts sorted (PR-blanched). The peanuts from the different processing methods were packaged in the four different packaging systems, each pack weighing 100-g, and stored in an incubator with controlled temperature of 30 ± 1 °C and humidity of 85% (regulated by saturated potassium chloride salt solution). The hermetic bags used were a special type of polyethylene storage bag (Super Grain-bag III, Grain Pro. Inc., Concord, MA, USA), with an oxygen transmission rate of 4.28 cc/m^2/day and water vapor transmission rate of 2.14 g/cm^2/day. The hermetic packs and polypropylene woven sacks (Sandbag Depot, Perth, Australia) used for the experiment had the same size of 17.8 cm × 20.3 cm. Oxygen absorbers (Oxyfree®, Marietta, GA, and USA) were used in one group of the packaging systems that were designated as 'HPO'. The treatments were analyzed for fungal growth, aflatoxin level, peroxide value, and *p*-Anisidine value at the time of their termination.

Fungal growth measurement

Measurement of fungal growth was based on the colony plate count method described by Dorner (2002). Peanut-water slurry was prepared by grinding shelled peanuts with an equal weight of distilled water for 7 min in a food processor. Some of the original slurry was kept and used for aflatoxin analysis and oil extraction. For quantification of *A. flavus* NRRL 3357, 50 g of sub-sample of the slurry was transferred to an autoclaved, stainless steel blender jar and 50 ml of sterilized, distilled water was added. The diluted slurry was blended for additional 1 min at low speed. After that, serial dilutions were spread on modified Dichloran-Rose Bengal medium (DRBA). The agar plates were incubated for 3 days at 37 °C and then colonies formed were counted manually.

Aflatoxin testing (ELISA test)

Post-storage peanut samples were analyzed for total aflatoxin as described by Dorner (2002). For quantification of total aflatoxin, a separate 10-g subsample of original slurry, as prepared for the mold or fungi count above, was transferred into a clean Falcon tube, 17.5 ml methanol was added to adjust the final methanol: water ration to 70:30, and tube was vortex mixed for 2 min. After that, aflatoxin content characterization was performed by the ELISA test (Agra Quant Total Aflatoxin Test Kit, Romer Labs) as the manufacturer described. A prepared mixture of peanut and 70% methanol was filtered through Whatman No#1 paper and the extract was directly mixed with enzyme-conjugated aflatoxin. The aflatoxin in extracted samples and the aflatoxin in enzyme-conjugated aflatoxin (provided by manufacturer) compete for the free binding sites when the mixture is loaded into anti-body coated micro-well. Presence of the substrate develops a blue color inversely proportional to the aflatoxin concentration in samples. Adding a stop solution changes the blue to a yellow color. This can be read using a dual wavelength (450 and 630 nm) micro-well reader (Model: EL312e, Bio-Tek Instruments Inc., Winooski, VT, USA).

Oil extraction

Methods of oil extraction followed Lee et al. (2010). Total lipids were extracted by mixing chloroform and methanol with each sample in a proportion of 1:1 v/v. This is a slight modification of the method of Bligh and Dyer (1959). Twenty-four grams (24 g) of the original peanut slurry

(having a water: peanut content with a ratio of 1:1 v/g) that was prepared for the fungal growth was used for the oil extraction. The slurry was transferred to Erlenmeyer flask and 15 ml of chloroform and 30 ml of methanol were added. The mixture was vortexed for 1–2 min. Then another 15 ml of chloroform was added and vortexed. Afterwards, 15 ml of distilled water was added and vortexed. The mixture was centrifuged at 1000 rpm for 5 min and the bottom phase of chloroform solution was collected using a pipette. The chloroform in the bottom phase was evaporated with a vacuum evaporator (Model R-3000; Buchi, rotavapor, Newcastle, USA) operated at 40 °C for 15 min. The lipid was transferred to centrifuge tubes, flushed with nitrogen, and stored at − 20 °C until it was analyzed.

Peroxide value (PV) measurement

The peroxide values of extracted oil samples were determined using the American Oil Chemists' Society (1998) official method Cd 8-53. Briefly, about 3-g of oil were put in a 125 ml glass Erlenmeyer flask; 30 ml acetic acid-chloroform solution (3:2, v/v) was added and shaken. Then 0.5 ml saturated potassium iodide solution was added, and swirled gently for exactly 1 min; the flask was stoppered and shaken vigorously to liberate the iodine from the organic layer. Starch indicator (1 ml) was added into the mixture and then titrated with 0.1 normality of sodium thiosulfate until the blue grey color disappeared. The volume of the titrant was recorded to the nearest 0.01 ml.

The peroxide value (milliequivalents peroxide/1,000 g) was computed using the following equation:

$$PV = \frac{(S - B) \times N \times 1000}{m} \quad (2)$$

where B is the titration of blank (ml), S is the titration of sample (ml), N is the normality of the sodium thiosulfate solution and m is the weight of the sample.

p-Anisidine value (*p*-Av) measurement

The amount of non-volatile aldehydes (principally 2-alkenals) in the oil was measured using *p*-Anisidine value by following AOCS official method Cd 18-90 (The American Oil Chemists' Society 1998). About 1 g of the oil sample was transferred into a volumetric flask, and mixed with 24 ml Iso-octane. The absorbance of this solution (Ab) was measured at 350 nm with a spectrophotometer, using Iso-octane as the blank. Then 5 ml of the mixture was measured and the blank was transferred separately into a new test tube. 1 ml of *p*-Anisidine was added to each test tube and incubated for 10 min. The absorbance (As) was recorded and the *p*-Anisidine value were calculated using the formula below:

$$pAV = \frac{2.5(1.2 \times As - Ab)}{m} \quad (3)$$

where As is the absorbance of the fat solution after reaction with the *p*-Anisidine reagent, Ab is the absorbance of the solution of the fat, and m is the mass of the test portion (g).

Statistical analysis

Data were analyzed using Analysis of Covariance (ANCOVA) in JMP-PRO version 13 (SAS Institute Inc., Cary, N.C., USA) treating time (weeks) as a continuous variable to adjust for least square estimates of treatment and interaction means of pre-storage treatments and packaging system. After examining the ANCOVA models for distribution of residuals, we determined that aflatoxin production and fungal growth had non-constant variance and non-normal residuals. We therefore log-transformed aflatoxin production log (x) and fungal growth log (1 + x) and refit the models. Peroxide and *p*-Anisidine values were not transformed. All four models met assumptions of the ANCOVA. Least square means of all the treatment combinations were compared using Turkey-Kramer test with corrected $\alpha = 0.05$ when a significant F-value was obtained.

Results and discussions

Fungal growth

Three of the pre-treatment methods (Raw-Cl, PR-blanched, PRN-blanch) resulted in little or no fungal growth regardless of the packing systems applied. Raw-Cl samples showed very little *Aspergillus* presence (mean of 6.91 CFU/g), because samples were sorted (infested, damaged, and discolored were removed) and partially sterilized under ultra violet (UV) light before storage studies. UV light is known to be good for killing microorganisms; it is the best method for disinfecting surfaces (Meechan and Wilson 2006). It is likely that UV was unable to kill possible fungi present inside the peanuts. Also, exposing peanuts to 145 °C for 10 min for partial roasting may have killed the fungi on the surface of peanuts. There were no significant differences ($p > 0.05$) in fungal growth for the PR-blanch, PRN-blanch, or Raw-Cl peanuts packaged in all the four packaging systems. Similarly, the fungal growth on Raw-Inf samples stored in three hermetic systems did not increase significantly ($p > 0.05$) over the 14 weeks of storage. Although the samples had *Aspergillus flavus* 3357 introduced and were

Table 1 Least square mean ± SE for fungi growth, and aflatoxin of Raw clean—Raw-Cl, Raw inoculated—Raw-Inf, partial-roasted blanch—PR-B and partial-roasted-not-blanched—PR-NB samples in the polypropylene woven sacks—PS, hermetic pack—HP, hermetic pack with oxygen absorber—HPO, hermetic pack vacuum sealed—HPV

Treatment/package	HP	HPO	HPV	PS	Mean
Fungi growth/CFU/g					
Raw-Cl	33.03 ± 0.82^{cde}	0.90 ± 0.82^{efg}	1.64 ± 0.82^{efg}	21.74 ± 0.82^{cdef}	6.91 ± 0.35^{B}
Raw-Inf	1046.74 ± 0.82^{b}	14.55 ± 0.82^{cdefg}	57.82 ± 0.82^{bcd}	$80{,}116.51 \pm 0.82^{a}$	525.38 ± 0.35^{A}
PR-B	0.69 ± 0.82^{efg}	0.17 ± 0.82^{fg}	1.58 ± 0.82^{efg}	88.16 ± 0.82^{bc}	2.77 ± 0.35^{B}
PR-NB	2.94 ± 0.82^{defg}	0.05 ± 0.82^{fg}	1.04 ± 0.82^{efg}	22.70 ± 0.82^{cdef}	3.62 ± 0.35^{B}
Overall-mean	21.06 ± 0.35^{B}	1.46 ± 0.35^{B}	4.37 ± 0.35^{B}	248.11 ± 0.35^{A}	
Aflatoxin production Levels/PPb					
Raw-Cl	6.96 ± 1.09^{bcd}	6.86 ± 1.09^{bc}	6.34 ± 1.09^{cd}	7.32 ± 1.09^{bc}	6.86 ± 1.04^{B}
Raw-Inf	10.61 ± 1.09^{b}	6.10 ± 1.09^{cde}	6.80 ± 1.09^{bcd}	18.40 ± 1.09^{a}	9.49 ± 1.04^{A}
PR-B	5.21 ± 1.09^{b}	3.99 ± 1.09^{e}	5.07 ± 1.09^{cde}	4.68 ± 1.09^{de}	4.71 ± 1.04^{C}
PR-NB	5.63 ± 1.09^{cde}	6.00 ± 1.09^{cde}	6.01 ± 1.09^{cde}	6.33 ± 1.09^{cd}	5.98 ± 1.04^{B}
Overall-mean	6.82 ± 1.04^{AB}	5.62 ± 1.04^{C}	6.02 ± 1.04^{BC}	7.95 ± 1.04^{A}	

Levels not connected by same letter case are significantly different. $p < 0.05$

stored under favorable environmental conditions, there was statistically insignificant growth because this hermetic system kills insects, mite pests, and prevents aerobic fungal growth (Weinberg et al. 2008). These results are comparable to the findings of Navarro et al. (2012) who reported that peanuts stored under controlled atmospheric conditions with 99% CO_2 concentration were able to suppress fungal growth below 97 CFU/g. Conversely, fungal growth in the peanut samples in polypropylene woven sacks displayed dormancy for about 6 weeks. During the next 8 weeks, the growth increased exponentially and recorded an average of 8.01×10^4 CFU/g. The lag phase for fungal growth was longer (6 weeks), compared to less than 7 days for peanuts with water activity of 0.92 and more (Ellis et al. 1994); and this could be attributed to the relatively low moisture content or water activity in the present study.

In case of Raw-Inf samples, the mean value of 8.01×10^4 CFU/g fungal concentration was recorded in samples stored in the polypropylene woven sacks; and it was significantly different from that of the other three packages (1.05×10^3 CFU/g for HP, 14.55 CFU/g for HPO, and 57.82 CFU/g for HPV; Table 1). The hermetic bags were able to reduce the fungal growth of the raw inoculated samples above 98% and the partial roasting was also able to eliminate the growth of fungi by killing the fungi present (Table 2).

Aflatoxin production

The aflatoxin production results from the combined effects of Raw-Cl, Raw-Inf, PR-blanch, and PRN-blanch peanut samples packaged in the PS, HP, HPO and HPV throughout the fourteen (14) weeks of storage are shown in Fig. 1. The Raw-Cl, PR-blanched, and PRN-blanch peanut samples had relatively lower aflatoxin mean values than Raw-Inf samples (Table 1). This could be because, as explained earlier, the aflatoxin-producing fungi (*Aspergillus flavus* NRRL 3357) were destroyed by the UV radiation and roasting used. Early detection and elimination of aflatoxin-producing fungi are critical to preventing this mycotoxin from entering the food chain.

Statistically, there were no significant differences in aflatoxin production in all the four packaging systems for the entire storage period for the three processing methods (Raw-Cl, PR-blanched and PRN-blanch). Although the aflatoxin levels did not increase over the fourteen weeks of storage (Fig. 1), it is obvious that there were small doses of aflatoxin in the peanut samples used, which were not visible enough to be sorted out before the study. The aflatoxin production for the raw inoculated samples in the hermetic bags were lower (HP = 10.61 ppb, HPO = 6.10 ppb and HPV = 6.80 ppb) than for the same samples in polypropylene woven sacks (38.26 ppb) at the 14th week of storage. This could be attributed to the hermetic bags helping to reduce oxygen levels. Since oxygen is essential for aflatoxin production in aflatoxigenic fungi, its absence in the packages (hermetic packaging) will minimize fungal growth (Paramawati et al. 2006) and hence aflatoxin production. Ellis et al. (1993) also reported that aflatoxin production by *A. flavus* was reduced to safe and acceptable levels (< 20 ppb) by reducing oxygen levels in packaging systems.

Overall, inoculated peanut samples in polypropylene woven sacks recorded the highest aflatoxin levels. Although all treatment combinations were stored under favorable environmental conditions (30 ± 1 °C, water

Table 2 Percentage reduction of fungi growth, aflatoxin, peroxide value and *p*-Anisidine values for the four peanut processing methods (raw clean—Raw-Cl, raw inoculated—Raw-Inf, partial-roast-blanched—PR-B, partial-roast-not-blanched—PR-NB) in three packaging systems (polypropylene woven sacks—PS, hermetic pack—HP, hermetic pack + O_2—HPO, hermetic-pack-vacuumed—HPV packaging) with respect to the raw-inoculated samples in polypropylene woven sacks

Pre-storage treatments and packaging	Fungi growth (%)	Aflatoxin production (%)	Peroxide-value (%)	*p*-Anisidine value (%)
Raw-Cl/HP	100.0	62.2	65.2	67.1
Raw-Cl/HPO	100.0	62.7	71.7	69.8
Raw-Cl/HPV	100.0	65.5	69.5	68.5
Raw-Cl/PS	100.0	60.2	69.3	64.5
Raw-Inf/HP	98.7	50.6	62.5	49.5
Raw-Inf/HPO	100.0	66.8	60.6	57.5
Raw-Inf/HPV	99.9	63.0	0.0	56.13
Raw-Inf/PS	0.0	0.0	0.0	0.0
PR-B/HP	100.0	73.3	62.3	59.0
PR-B/HPO	100.0	78.3	67.3	66.0
PR-B/HPV	100.0	72.4	64.8	60.1
PR-B/PS	99.8	74.6	38.1	48.5
PR-NB/HP	99.9	69.4	65.7	58.1
PR-NB/HPO	100.0	67.33	66.3	62.0
PR-NB/HPV	100.0	67.33	63.3	59.6
PR-NB/PS	99.8	63.4	42.2	40.7

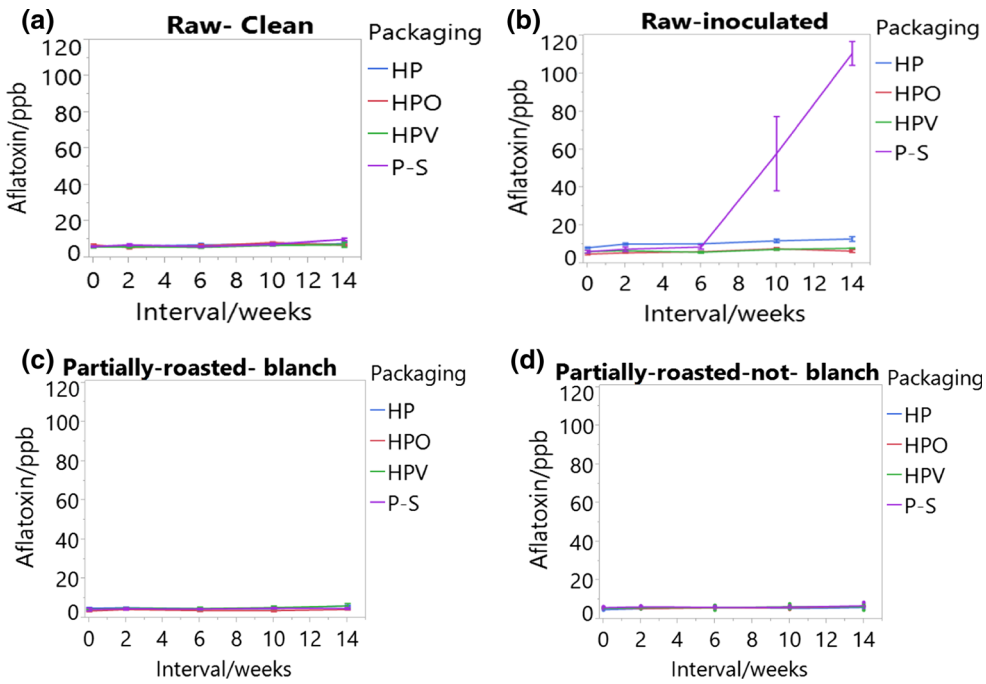

Fig. 1 Aflatoxin production results of the raw clean (**a**), raw inoculated (**b**), partially roasted blanched (**c**) and partially roasted not blanched (**d**) peanut treatments in the four packaging (PS, HP, HPO, HPV) system

activity 0.85 ± 0.02, and humidity 85%) for aflatoxin production, the presence of oxygen may have accounted for the difference. This can be compared to the study of Vaamonde et al. (2006) who recorded the highest aflatoxin value of 4450 ppb in peanuts stored with 0.86 water activity at 30 °C for 28 days. It was observed that all the hermetic bags were able to reduce aflatoxin production of the raw inoculated samples by 50.6% (HP), 63.0% (HPV) and 66.8% (HPO) and partial roasting and blanching was also able to reduce aflatoxin production by above 72% (Table 2). From these data, although the fungi were suppressed in the hermetic bags, the aflatoxin reduction was

quite low and there is a possibility that, there were small doses of aflatoxin in the peanuts used for the study which was not detected easily by sorting.

Peroxide value

Peroxide value, as a measure of lipid oxidation (quality), showed a slight increase in oil samples extracted from all the treatment combinations stored over the fourteen-week period (Fig. 2). During lipid oxidation, there is a web of complex oxidation processes involved in the generation of oxidative degradation products from their precursor fatty acids (Yun and Surh 2012). There are intrinsic factors, such as antioxidants, prooxidants, and water in the oils, that might simultaneously operate in a complementary or opposite way to affect the fatty acids (Kamal-Eldin; 2006; Merrill et al. 2008) and, with a relatively high moisture content of 10 ± 1% in the samples, this could have been a contributing factor to this increasing trend of the peroxide value during the storage.

The increase in peroxide values for raw inoculated peanut samples packaged in polypropylene woven sacks was the highest among all the treatments and had a mean value of 30.74 meq/kg compared to the least mean value of 8.71 meq/kg for raw clean samples in hermetic bags with oxygen absorber (Table 3). This could be explained by the free oxygen passage in the package as the presence of oxygen in the sample can increase lipid oxidation, thus affecting the fatty acid composition of the lipids (Ul-Hassan and Ahmed 2012). External factors, such as light, elevated temperatures, and oxygen contribute to the generation of oxidative degradation products from their precursor fatty acid (Kamal-Eldin 2006; Merrill et al. 2008). Furthermore, fungal growth resulting in aflatoxin production can increase oxidation. This could be a result of the release of enzymes (lipase) by the aflatoxin-producing fungi which hydrolyze the fatty acids in peanuts which, in turn, initiates lipid oxidation leading to rancidity (Lam and Proctor 2003). Thus, microbial growth is correlated with free fatty acid (FFA) formation. There was a very strong correlation ($r^2 = 0.87$, RMSE = 7.26, $p = 0.0001$) between aflatoxin production and quality (peroxide value) for raw inoculated samples. Studies have shown simultaneous increases in FFA levels and microbial growth (Lam and Proctor 2003).

At $\alpha = 0.05$, the peroxide values of all the processing methods were significantly different from one another, apart from the PR-blanched and PRN-blanch which were not significantly different ($p = 0.77$). Similarly, when the peroxide values of the four packaging systems were compared with one other, they were individually distinct except that HP and HPV were not significantly different ($p = 0.20$); nor did HPO differ from HPV ($p = 0.30$). Although it is difficult to isolate a single factor that causes lipid oxidation, it was expected that all the partially roasted samples would have a lower oil quality (i.e., higher peroxide value) because they were exposed to elevated temperature during roasting. Comparing all samples to raw inoculated samples in polypropylene woven sacks, which had the worst peroxide value recorded (see Table 2) with the hermetic packaging, it was possible to reduce quality deterioration in terms of peroxide value in the range of

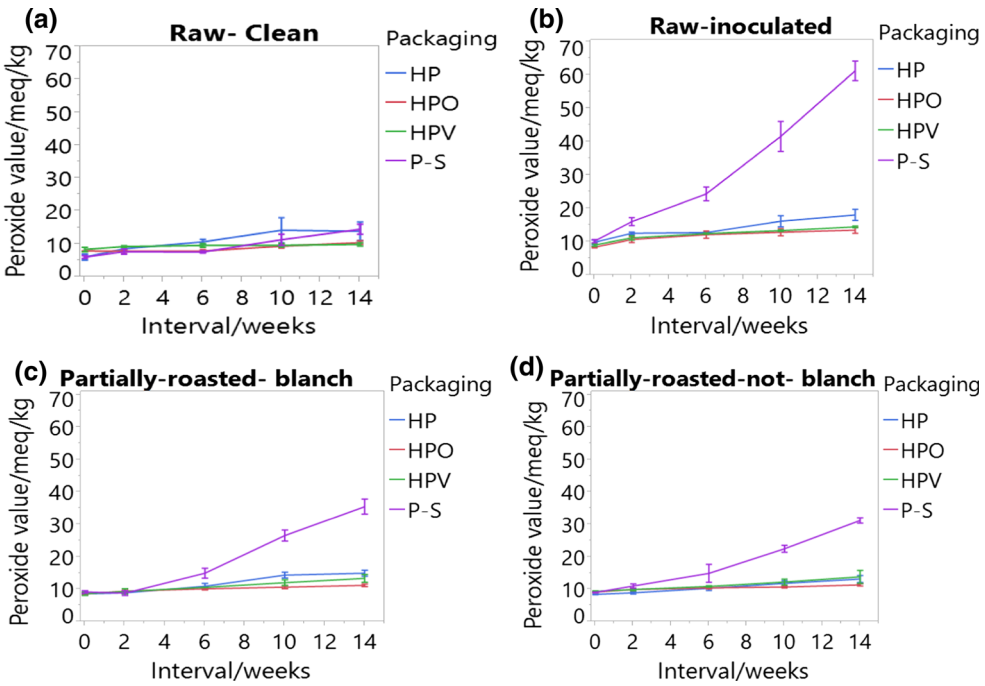

Fig. 2 Peroxide value results for **a** raw-clean, **b** raw inoculated, **c** partially roasted blanched and **d** partially roasted not blanched peanut samples in the four packaging (PS, HP, HPO, HPV) systems

Table 3 Least square mean ± SE for peroxide and *p*-Anisidine values of raw clean—Raw-Cl, raw inoculated—Raw-Inf, partial-roast blanch—PR-B and partial-roast-not-blanch—PR-NB samples in the polypropylene woven sacks—PS, hermetic pack—HP, hermetic pack with oxygen absorber—HPO, hermetic pack vacuumed—HPV

Treatment/package	HP	HPO	HPV	PS	Mean
Peroxide value (meq/kg)					
Raw-Cl	10.65 ± 1.34^{cd}	8.71 ± 1.34^{d}	9.37 ± 1.34^{d}	9.44 ± 1.34^{d}	9.55 ± 0.67^{C}
Raw-Inf	13.77 ± 1.34^{bcd}	11.53 ± 1.34^{bcd}	12.12 ± 1.34^{cd}	30.74 ± 1.34^{a}	17.07 ± 0.67^{A}
PR-B	11.59 ± 1.34^{bcd}	10.06 ± 1.34^{cd}	10.83 ± 1.34^{cd}	19.04 ± 1.34^{b}	12.88 ± 0.67^{B}
PR-NB	10.56 ± 1.3^{cd}	10.35 ± 1.3^{cd}	11.29 ± 1.34^{bcd}	17.77 ± 1.34^{bc}	12.49 ± 0.67^{B}
Overall-mean	11.69 ± 0.67^{B}	10.16 ± 0.67^{B}	10.90 ± 0.67^{B}	19.25 ± 0.67^{A}	
p-Anisidine value (meq/Kg)					
Raw-Cl	3.46 ± 0.35^{bcd}	3.18 ± 0.35^{d}	3.32 ± 0.35^{d}	33.74 ± 0.35^{ecd}	3.43 ± 0.18^{C}
Raw-Inf	5.32 ± 0.35^{abc}	4.48 ± 0.35^{bcd}	4.62 ± 0.35^{bcd}	10.53 ± 0.35^{a}	56.24 ± 0.18^{a}
PR-B	4.32 ± 0.3^{dbcd}	3.58 ± 0.35^{fgh}	4.20 ± 0.35^{cd}	5.42 ± 0.35^{a}	4.38 ± 0.18^{B}
PR-NB	4.41 ± 0.35^{cd}	4.0 ± 0.35^{cd}	4.25 ± 0.35^{cd}	6.24 ± 0.35^{ab}	4.72 ± 0.18^{BC}
Overall-mean	4.38 ± 0.18^{B}	3.81 ± 0.18^{C}	4.10 ± 0.18^{C}	6.48 ± 0.18^{A}	

Levels not connected by same letter case are significantly different. $p < 0.05$

62.3% (PR-blanched in HP) to 67.27% (PR-blanched in HPO) for all the partially roasted (blanch and not-blanch) samples. All the peanut samples were stored under the same environmental conditions.

p-Anisidine value

p-Anisidine value assay measures non-volatile secondary oxidation products in oils. During secondary oxidation, unstable molecules decompose readily to form a myriad of products such as aldehydes, ketones, alcohols and hydrocarbons, etc. (Shahidi and Wanasundara 1998). These impart unpleasant flavors and odors to fats and oils (lipids) in food. As in the peroxide values, the *p*-Anisidine values from the three samples from hermetic packages showed a slight increase over the 14-week storage period for the Raw-Cl, PR-blanched and PRN-blanch samples (Fig. 3). Although hermetic packages are airtight, other factors such as light and temperature (Kamal-Eldin 2006; Merrill et al. 2008) were present, explaining the slight increases in the *p*-Anisidine values. There are many procedures for controlling the rate and extent of lipid oxidation in food, but the addition of antioxidants has proven to be the most effective (Barriuso et al. 2013). This may be the reason why samples

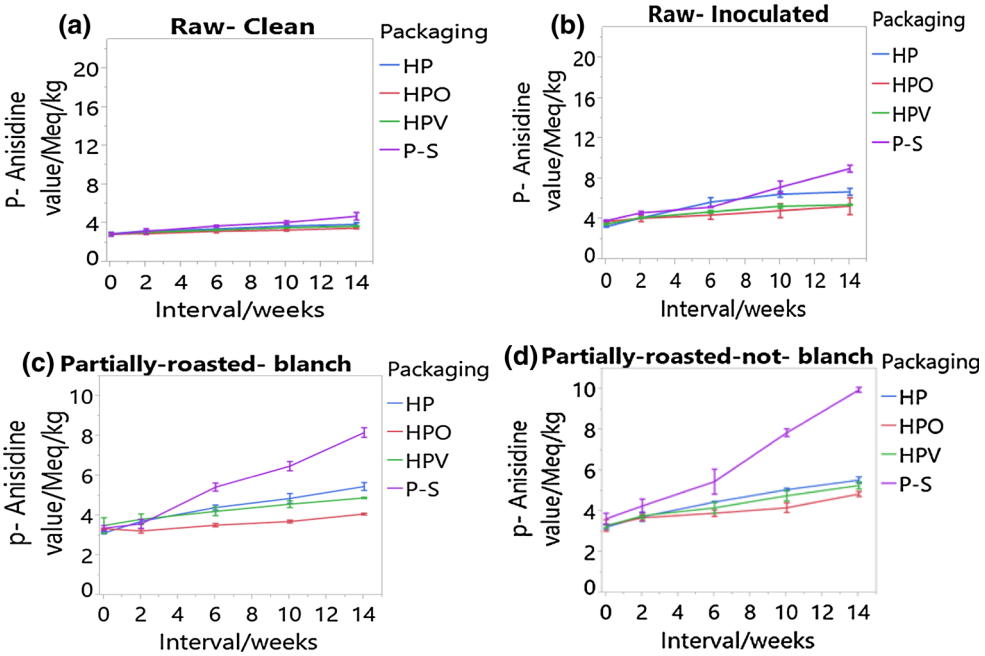

Fig. 3 Effects pretreatments [raw clean (**a**), raw inoculated (**b**), partially roasted blanched (**c**) and partially roasted not blanched (**d**)] and packaging (PS, HP, HPO, HPV) of *p*-Anisidine results

in hermetic bags with oxygen absorbers recorded the lowest mean value of 3.85 meq/kg; while polypropylene woven sacks recorded 5.30 meq/kg (Table 2). We also observed that, the raw inoculated samples in polypropylene woven sacks sampled from week 10 onwards had a bad odor. This is attributed to fungi growth because microbial growth is correlated with free fatty acid formation. The products from the secondary oxidation have the potential to produce undesirable sensorial and biological effects (Márquez-Ruiz et al. 2007). The results also showed a strong correlation ($r^2 = 0.93$, p value < 0.0001, RMSE $= 0.55$) between aflatoxin production and quality (p-Anisidine value) for the Raw-Inf samples. The p-Anisidine value for all four pre-storage treatments were significantly different from one another at ($p < 0.05$), with the overall mean values recording 3.43 meq/kg for Raw-Cl, 6.24 meq/kg for Raw-Inf, 4.38 meq/kg for PR-blanched, and 4.72 meq/kg for PRN-blanch (Table 3).

Overall, the raw inoculated samples in polypropylene woven sacks recorded the highest p-Anisidine value. Comparing the p-Anisidine values of raw inoculated samples to all the other treatment combinations, the raw clean samples in the three hermetic bags were able to reduce the quality deterioration by 67.14% (HP), 68.47% (HPV) and 69.80% (HPO), followed by partially roasted blanched samples in hermetic bags with oxygen absorbers recording 66% (Table 2).

Conclusion

This study has shown that it is best to clean peanuts either by sorting or disinfecting the nuts (finding ways to kill the fungi on the product) before storage. There were no significant fungal growth, aflatoxin production and lipid oxidation observed in Raw-Cl, PR-blanch, and PRN-blanched peanuts over the storage period compared to the infested (Raw-Inf) samples. Overall, although PR-blanch peanuts produced lower level of aflatoxin production over the storage period, Raw-Cl samples were the best in terms of quality maintenance, followed by PR-blanch peanut samples. To achieve lower aflatoxin values during storage for shelled peanuts, it is best to partially roast, blanch and then sort out the infested ones.

Hermetic packages were robust in controlling fungal growth and aflatoxin as well as maintaining quality, regardless of the type of pre-treatment method used. Of all the four packaging systems, the hermetic bags with oxygen absorbers were the best. Therefore, it is recommended that peanuts be stored hermetically with zero-oxygen in the package. It is recommended that a study be conducted to find the effect of storage time on fungal growth and aflatoxin production, with varying water activity levels under controlled optimum environmental conditions (for temperature and humidity levels) with specific emphasis on the lag phase of the fungal growth.

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References

Akbas MY, Ozdemir M (2006) Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. J Sci Food Agric 86(13):2099–2104

Barriuso B, Astiasarán I, Ansorena D (2013) A review of analytical methods measuring lipid oxidation status in foods: a challenging task. Eur Food Res Technol 236(1):1–15

Basaran P, Akhan Ü (2010) Microwave irradiation of hazelnuts for the control of aflatoxin producing *Aspergillus parasiticus*. Innov Food Sci Emerg Technol 11(1):113–117

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917

Dorner JW (2002) Simultaneous quantitation *of Aspergillus flavus/A. parasiticus* and Aflatoxins in Peanuts. J AOAC Int 85(4):911–916

Ellis WO, Smith JP, Simpson BK, Khanizadeh S, Oldham JH (1993) Control of growth and aflatoxin production of *Aspergillus flavus* under modified atmosphere packaging (MAP) conditions. Food Microbiol 10:9–21

Ellis WO, Smith J, Simpson B, Ramaswamy H, Doyon G (1994) Growth and aflatoxin production by *Aspergillus flavus* in peanuts stored under modified atmosphere-packaging (MAP) conditions. Int J Food Microbiol 22:173–187

Garcia D, Ramos AJ, Sanchis V, Marin S (2011) Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus Ochraceus* and *Aspergillus parasiticus*. Food Microbiol 28(3):406–417

Hell K, Cardwell KF, Setamou M, Poehling HM (2000) The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. J Stored Prod Res 36:365–382

Jalili M, Jinap S, Noranizan A (2010) Effect of gamma radiation on reduction of mycotoxins in black pepper. Food Control 21:1388–1393

Kaaya AN, Kyamuhangire W (2006) The effect of storage time and agro ecological zone on mold incidence and aflatoxin contamination of maize from traders in Uganda. Int J Food Microbiol 110:217–223

Kamal-Eldin A (2006) Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. Eur J Lipid Sci Technol 58:1051–1061

Kumar A, Shukla R, Singh P, Anuradha Y, Dubey NK (2010) Efficacy of extract and essential oil of Lantana indica Roxb. Against food contaminating molds and aflatoxin B1 production. Int J Food Sci Technol 45:179–185

Lam HS, Proctor A (2003) Lipid hydrolysis and oxidation on the surface of milled rice. J Am Oil Chem Soc 80(6):563–567

Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM (2010) Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 101(1): S75–S77

Magnussen A, Parsi MA (2013) Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol 19(10):1508

Márquez-Ruiz G, Holgado F, García-Martínez MC, Dobarganes MC (2007) A direct and fast method to monitor lipid oxidation progress in model fatty acid methyl esters by high-performance size-exclusion chromatography. J Chromatogr A 1165(1):122–127

Meechan PJ, Wilson C (2006) Use of ultraviolet lights in biological safety cabinets: a contrarian view. Appl Biosaf 11(4):222–227

Merrill LI, Pike OA, Ogden LV, Dunn ML (2008) Oxidative stability of conventional and high-oleic vegetable oils with added antioxidants. J Am Oil Chem Soc 85(8):771–776

Mutegi C, Wagacha G, Christie M, Kimani J, Karanja L (2013) Effect of storage conditions on quality and aflatoxin contamination of peanuts (*Arachis hypogaea* L.). Int J Agric Sci 3(10):746–758

Navarro H, Navarro S, Finkelman S (2012) Hermetic and modified atmosphere storage of shelled peanuts to prevent free fatty acid and aflatoxin formation. Integr Prot Stored Prod IOBC-WPRS Bull 81:183–192

Official AOCS (1998) Tentative methods of the American Oil Chemists Society. AOCS, Chicago

Ogunsanwo BM, Faboya OOP, Idowu OR, Lawal OS, Bankole SA (2004) Effect of roasting on the aflatoxin contents of Nigerian peanut seeds. Afr J Biotech 3:451–455

Paramawati R, Widodo P, Handaka BU (2006) The role of postharvest machineries and packaging in minimizing aflatoxin contamination in peanut. Indones J Agric Sci 7(1):15–19

Park DL, Lee LS, Price RL, Pohland AE (1987) Review of the decontamination of aflatoxins by ammoniation: current status and regulation. J Assoc Off Anal Chem 71(4):685–703

Shahidi F, Wanasundara UN (1998) Omega-3 fatty acid concentrates: nutritional aspects and production technologies. Trends Food Sci Technol 9(6):230–240

Ul-Hassan F, Ahmed M (2012) Oil and fatty acid composition of peanut cultivars grown in Pakistan. Pak J Bot 44:627–630

Vaamonde G, Patriarca A, Pinto VEF (2006) Effect of water activity and temperature on production of aflatoxin and cyclopiazonic acid by *Aspergillus flavus* in peanuts. Adv Exp Med Biol 571:225–235

Weinberg ZG, Yan Y, Chen Y, Finkelman S, Ashbell G, Navarro S (2008) The effect of moisture level on high-moisture maize (*Zea mays* L.) under hermetic storage conditions in vitro studies. J Stored Prod Res 44(2):136–144

Yazdanpanah H, Mohammadi T, Abouhossain G, Cheraghali AM (2005) Effect of roasting on degradation of aflatoxins in contaminated pistachio nuts. Food Chem Toxicol 43:1135–1139

Yun JM, Surh J (2012) Fatty acid composition as a predictor for the oxidation stability of Korean vegetable oils with or without induced oxidative stress. Prev Nutr Food Sci 17(2):158–165