Effect of Alkali Refining on Removal of Aflatoxins in Groundnut Oil and Quality Evaluation

Atif A. A. Yassin[1*]; Reem A. M. A. Magzoub[1]; Nada A. A. Abdelrahman[2] and Awad M. Abdel-Rahim[3]

[1.] National Oilseed Processing Research Institute (NOPRI), University of Gezira
[2.] Food Engineering Department, Faculty of Engineering and Technology, University of Gezira
[3.] Faculty of Science, University of Gezira

*** Corresponding author. E-mail address:** atifaay@hotmail.com

ABSTRACT

The present study was investigated the effect of alkali refining on the removal of aflatoxins (AFs) in groundnut oil. Groundnut oil samples were collected from different expellers in Medani and Khartoum cities in Sudan. The samples were investigated for their fungal growth using potato dextrose agar (PDA) media and quantitation of the toxins was accomplished by the thin layer chromatography (TLC) technique. The results show that most of the oil samples were affected by *A. flavus* and other fungi as well as contaminated with AFs. The findings show that the alkali refining processes of the groundnut oil slightly effect on the removal of AFs in groundnut oil. In addition, there was significant difference in the physicochemical properties of the groundnut oil after treatment via alkali refining, these include the moisture content, peroxide value, acid value as well as the free fatty acids. On the other hand, there were no significant changes in the refractive index before and after alkali refining.

Keywords: Groundnut oil, aflatoxins, *A. flavus*, alkali refining

INTRODUCTION

The presence of Aflatoxins (AFs) in edible oils is one of the issues of food safety since they are the potential source of health hazards (Lupo *et al.*, 2016; Grag *et al.*, 2013). AFs are secondary metabolite produced mainly by *A. flavus* and *A. parasiticus*. AFs are considered an unavoidable and naturally occurring contaminant of groundnut and groundnut oil (Bakhiet and Musa, 2011; Oliveira *et al.*, 2009). Low-dose consumption of foods contaminated with AFs for a long time causes chronic aflatoxicosis, resulting in cancer, suppression of immunological responses and other pathological conditions (Naz *et al.*, 2016). In developing countries, the incidence rates of liver cancer are two to ten times higher than those in developed countries due to AFs contaminated food intake. Furthermore, AFs contamination are leading to serious economic loss to the groundnut oil enterprises (Aiko and Mehta, 2015; Das, 2007).

Epidemiological studies and animal experiments showed that AFs have strong genotoxic, carcinogenic and immunotoxic effects on humans and animals (Zain, 2011). Therefore, many countries have legislated on the maximum levels of AFs in foods to reduce its harms to humans and animals. For instances, the European Commission (EC) has set stricter standards, which establishes that any products for direct human consumption, the maximum permissible concentration of AFB1 and total AFs should not be greater than 2 μgkg^{-1} and 4 μgkg^{-1}, respectively (European Commission-EC, 2010; Van de Perre *et al.*, 2015). Interestingly, the joint Food and Agriculture Organization (FAO)/ World Health Organization (WHO)/Codex Alimentarius Commission (CODEX) with the mandate of setting international standards not greater than 15 μgkg^{-1}. Likewise, the United States (US) regulations have specified the maximum acceptable limit of total AFs contamination in groundnut as 20 μgkg^{-1} (Codex Alimentarius, 1995; Food and Agriculture Organization FAO, 1997; World Health Organization WHO, 1998; Prietto *et al.*, 2015; Bhat and Reddy, 2017).

Oils and fats are found in natural products where levels of impurities and contaminants may vary with the type of raw materials in addition to external factors such as climate, soil, harvesting, storage and processing conditions (Bordin *et al.*, 2014). Groundnut oil or peanut oil (as it is known in some parts of the world) and sometimes known as arachis oil is commonly consumed in Sudan and other developing countries owing to its high nutritional content of unsaturated fatty acids and vitamins, pleasant flavor and cooking quality (Chang *et al.*, 2013). High contamination rates of AFs in groundnut oil have been reported in China (Mao *et al.*, 2016), India (Pereira *et al.*, 2014), Nigeria (Odoemelam and Osu, 2009) and other countries (Jalili, 2015).

AFs can be removed or eliminated from food products with the application of physical, chemical or biological methods. Physical processes involve separation of the contaminated parts, removal and inactivation of AFs by physical means, such as heat, cooking, roasting, and radiation (Grag *et al.*, 2013). Chemical treatment by using strong alkalis or oxidants such as (ammonia, ozone, chlorine and hydrogen peroxide) to break the structure of AFs. While, biological methods (e.g., fermentation, enzymes and microbial metabolization) showed the high efficiency and selectivity,

at the same time, these methods may be difficult to reutilize on a large scale (Mao *et al.*, 2016). Few methods have been reported for AFs degradation in groundnut oil. Shantha and Murthy (1977) stated that treatment of groundnut oil with ultraviolet (UV) light for 2 h destroyed 40 – 45% of AFs initially present in the oil. Diao *et al.* (2015) used UV irradiation (365 nm) to degrade AFB1 in groundnut oil, which was reduced by 86.08% within 10 min. Alkali refining is an important process in oil refining, which is used to remove free fatty acids (FFA) in vegetable oils. Parker and Melnick (1966) used alkali refining to reduce the AFs content in corn oil to a range of 10 – 14 $\mu g kg^{-1}$. The detoxifying process of AFs in groundnut oil has not been reported in detail and the safety of groundnut oil after being refined is still unknown. Thus, the aims of this study were to investigate the presence of *A. flavus*, microbial content and AFs contamination in groundnut oil obtained from different expellers in Sudan and to study the effect of alkali refining method on AFs removal as well as to evaluate the quality of groundnut oil after refining processes.

MATERIALS AND METHODS

1. Analysis of aflatoxins using TLC

Eight samples of Groundnut oil were obtained from different Expellers in Medani (M1, M2, M3 and M4) and Khartoum (K1, K2, K3 and K4) cities. The samples were investigated for their fungal growth using potato dextrose agar (PDA) media. Inoculated plates were incubated in an incubator at 37 °C. The plates were investigated daily for fungal growth (McDonald and Harkness, 1963). Colonies of the fungus, *A. flavus* green colour were detected and calculated as percentage. Contamination with fungi other than *A. flavus* was also calculated. AFs were determined before and after the alkali treatment using the methods described by Jones (1972) as follows; ten mL of groundnut oil were placed into a 250 mL flask. Then, 10 mL of distilled water were added and thoroughly mixed. After that, 100 mL chloroform was added. The flasks contents were shaken on a griffin shaker for 30 minutes to ensure good extraction before they filtered through a filter paper (24 cm). The chloroform was then evaporated to dryness in a water bath at 70 °C.

Quantitation of the toxins was accomplished by the thin layer chromatography (TLC) technique as follows; the pre-coated chromatographic papers were used and heated in an oven at 105 °C For 1 hour. After that they cooled in a dust free atmosphere for 30 minutes before being placed into a plate cabinet. The dried extracted samples were washed by 2 mL of chloroform. An amount of 5 to 25 µl of the solution was spotted on the prepared TLC paper by a micro syringe. The papers were then dried before being developed in diethyl ether solution in a chromatic tank. Next, they were allowed to dryness before they were redeveloped in a solution of a mixture of chloroform-methanol (97:3 v/v). Afterwards, the solution was allowed to move 10 cm above the base line of the paper. Subsequently, the papers were dried and examined in a dark room under ultra violet

light lamp (peat emission 366 mm, philiips Hp w 125 watts type) at 30 cm distance from the lamp (Diener and Davis, 1968; Jones, 1972).

2. Groundnut oil alkali refining

The chemical treatment by alkali refining was performed according to Gupta (2008). The samples were first heated to 60 ± 5 °C and then treated with phosphoric acid according to number of phospholipids in the crude oil. Then, the samples were heated to 40 ± 5° C. A caustic solution (sodium hydroxide) was added before it reacted with FFAs in the crude oil. Produced soap was separated from the refined oil in a primary refining centrifuge, the remaining soap was mostly removed from oil via water washing by centrifugation

3. Physical properties

3.1 Determination of moisture content (MC)

The moisture content was determined using the AFNOR method (Augustin *et al.*, 2015). The moisture content (*MC*) was expressed as percentage by mass using the formula below:

$$\text{MC } \% = \frac{\text{Mb-Md}}{\text{Mb-M}} \times 100 \qquad (1)$$

where *M*, *Mb* and *Md* are the mass of the Petri dish (g), Petri dish with the sample (g), and Petri dish with the dried sample (g), respectively.

3.2 Refractive index (RI)

The refractive index was determined by an automatic digital refractometer (Model ATAGO RX - 5000α) according to the AOCS Official Method Tp, 1a–64 at 20 °C (AOCS, 2009).

4. Chemical properties

4.1 Peroxide value (PV)

The AOAC Official Method 965.33 (AOAC, 2000) was followed to determine the PV of the oil samples. The peroxide value, *PV* (milliequivalent peroxide/kg sample) was calculated using the following equation:

$$\text{PV} = \frac{(\text{S-B})(\text{N})}{(\text{A})} \times 1000 \qquad (2)$$

where *S* is the $Na_2S_2O_3$ titration of the oil sample (mL), *B* is the $Na_2S_2O_3$ titration of the blank (mL), *N* is the normality of $Na_2S_2O_3$ and *A* is the weight of oil sample (g).

4.2 Acid value (AV) / Acidity

The AOCS Official Method Te 1a-64 reapproved 2009 (AOCS, 2009) was followed to determine the AV of the oil samples. The acid value, *AV* (mg KOH/g of FAs) was calculated using the following equation:

$$\text{AV} = \frac{(\text{S})(\text{M})}{(\text{A})} \times 56.1 \qquad (3)$$

where *S* is the volume of the titrant (mL), *M* is the molarity of NaOH solution and *A* is the mass of oil sample (g).

4.3 Free fatty acids (FFA)

The FFA was determined in accordance with the norms of AOCS Method Ca 5a-40 (AOCS, 2009). The *FFA* was calculated as percentage of oleic acid using the following equation:

$$\text{FFA } \% = \frac{(S)(M)}{(A)} \times 28.2 \qquad (4)$$

where *S* is the volume of the titrant (mL), *N* is the normality of potassium hydroxide solution and *A* is the mass of the oil sample (g).

5. Statistical analysis

All the experiments were conducted in triplicates under identical conditions. The data were subjected to analysis of variance (ANOVA) using SPSS software (IBM, PASW Statistics 19, USA). The results are presented as mean ± standard deviation (SD) and P values ≥ 0.05 were considered statistically insignificant.

RESULTS AND DISCUSSION

1. Fungal growth and AFs contamination

Table (1) shows the fungal growth and AFs contamination in groundnut oil samples results for alkali refining. As can be seen, most of the oil samples were found affected by *A. flavus and* other fungi as well as contaminated by AFs except M1 and K1 oil samples which were found to be free from incidence by *A. flavus* and AFs contamination. Moreover, *A. niger* was dominant in M1 oil samples. The high levels of AFs could be consequent to inappropriate storage. Thus, adopting internationally recommended harvest procedures at farm levels by implementing hazard analysis and critical control point (HACCP) procedures as well as adopting good agriculture and good manufacturing practices (GAP and GMP) might significantly reduce the AFs contamination in groundnut and its products. Some of the important criteria to be practiced include: handling of groundnut seeds without injury, drying to acceptable moisture and water activity levels, proper transportation and proper storage to prevent damp storage abuse and minimizing incidence by fungi. The results obtained agreed with previous work carried out by Elzupir *et al.* (2010) who reported the levels of total AFs in edible oil in Khartoum State are quite alarming. AFs contamination was detected in 80/81 samples (98.8%). Furthermore, the treatment of oil samples via alkali had slight change on elimination of AFs contamination. The measurement of AFs by TLC was qualitative analysis and therefore the quantitative analysis is very important to calculate the reduction percentage.

Table 1: Microbial content and AFs contamination of the groundnut oil

Sample name	***A. flavus* %**	***A. niger* %**	***Other fungi***	**Aflatoxins**	
				unrefined oil	**refined oil**

M_1	0	8	+	-	-
M_2	1	3	+	++	+
M_3	1	1	+	++	+
M_4	1	0	+	++	+
K_1	0	0	+	-	-
K_2	1	1	+	++	+
K_3	1	0	+	++	+
K_4	1	4	+	++	+

Note: Medani expellers (M1, M2, M3 and M4); Khartoum expellers (K1, K2, K3 and K4); other fungi (Rhizopus, mold and yeast); where (-) = not-detected; (+) = detected and (++) = high concentration

2. Physical properties

Table (2) illustrates the moisture content and refractive index of the groundnut oil samples before and after treatment. The moisture content was in the range (0.117 ± 0.003 - 0.525 ± 0.001) and (0.045 ± 0.002 - 0.125 ± 0.001) for the crude oil and refined oil samples, respectively. The moisture content of refined oil samples was reduced obviously within the recommended Codex Standards for edible oils (Sulieman *et al.*, 2013). The statistical analysis proved that there were insignificant differences in the RI of the oil samples. The RI of the samples was obtained range (1.4699 ± 0.0001 - 1.4710 ± 0.0001). This result is in agreement with results reported by El-Nakhlawy and Bakhashwain (2009) and Nkafamiya *et al.* (2010). The refractive index was no significant difference in the degrees of flow or thickness of all the oil at room temperature.

Table 2: Physical properties of groundnut oil before and after alkali treatment.

Sample name	**Moisture content (%)**		**Refractive index (n^{20}_D)**	
	Crude oil	**Refined oil**	**Crude oil**	**Refined oil**
M1	0.166 ± 0.002	0.066 ± 0.001	1.4710 ± 0.0001	1.4710 ± 0.0001
M2	0.525 ± 0.001	0.125 ± 0.001	1.4708 ± 0.0001	1.4708 ± 0.0001
M3	0.299 ± 0.001	0.105 ± 0.001	1.4709 ± 0.0001	1.4709 ± 0.0001
M4	0.117 ± 0.003	0.055 ± 0.003	1.4706 ± 0.0001	1.4706 ± 0.0001
K1	0.320 ± 0.003	0.115 ± 0.003	1.4699 ± 0.0001	1.4699 ± 0.0001
K2	0.415 ± 0.003	0.120 ± 0.002	1.4701 ± 0.0001	1.4701 ± 0.0001
K3	0.295 ± 0.002	0.101 ± 0.001	1.4702 ± 0.0001	1.4702 ± 0.0001
K4	0.129 ± 0.002	0.045 ± 0.002	1.4701 ± 0.0001	1.4701 ± 0.0001

Note: Medani expellers (M1, M2, M3 and M4); Khartoum expellers (K1, K2, K3 and K4); all values represent the means ± standard deviation; n = 3.

3. Chemical properties

Generally, alkali refining has noticeable effect on the chemical properties of groundnut oil. The variation of PV in crude and refined groundnut oil samples was clarified in Figure (1). The PV was significantly increased after refining process. Relatively high value was found in M4 sample,

while the lowest value was reported in K1 sample. The peroxide formation is slow at first during an induction period that may vary from few weeks to several months according to the particular oil and temperature (Olaposi and Adunni, 2010). The PV is an indicator of deterioration of oils and/or fats; the low PV indicated slow oxidation of oils according to Demian (1990). The increase in PV could be attributed to the oxidation of fatty acids due to several factors like high temperature, packing in containers impermeable to light and loose lock in addition to poor storage and poor handling (Zeng *et al*., 2010). This result was correlated with the result of Abd-El-Gawad (2009).

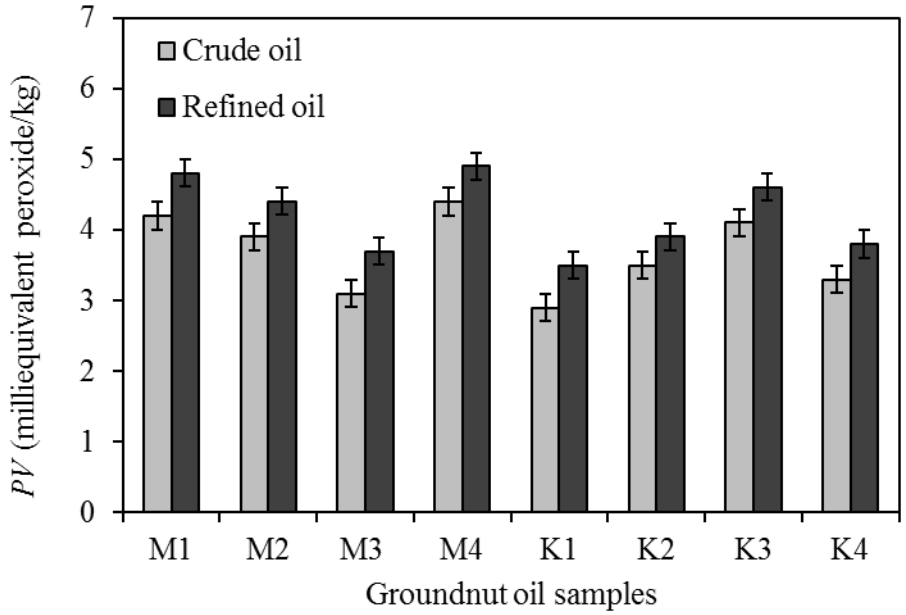

Figure 1: Peroxide value of crude and refined groundnut oil from different expellers

It was observed that after alkali refining, the acid value of groundnut oil samples from different expellers was decreased distinctly which improved the quality of groundnut oil (Figure 2). The maximum reduction of AV was in K4 oil sample (from 1.1 to 1.08 mg KOH/g of oil) in crude and refined oil, respectively. The result obtained agreed with previous work carried out by Kostik *et al.* (2013) and Shen *et al.* (2014).

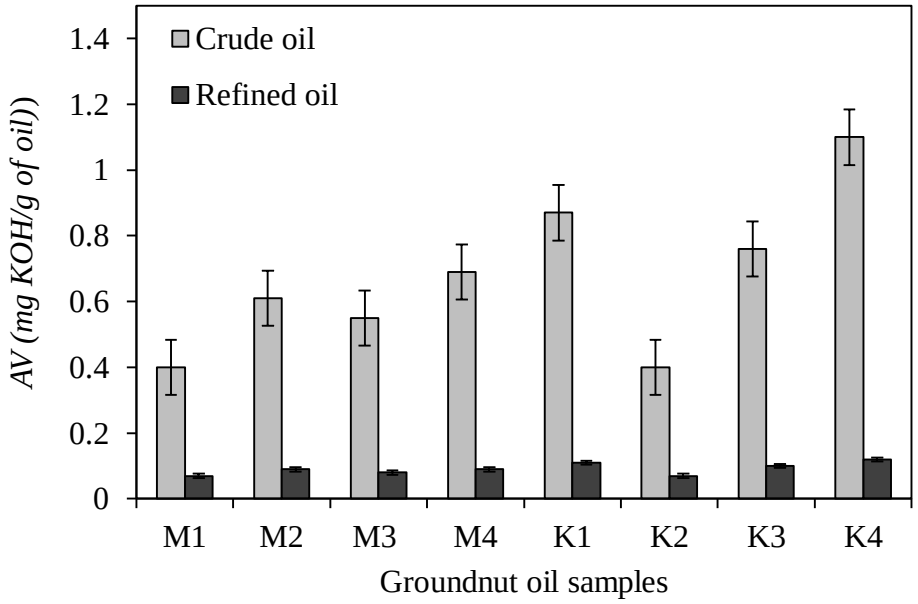

Figure 2: Acid value of crude and refined groundnut oil from different expellers

It was very interesting to find that after alkali refining the rate of free fatty acids was apparently decreased (Figure 3). The results demonstrate that the FFA of the samples were between (0.20 - 0.55%) for the crude oil and between (0.04 - 0.06%) for the refined oil. These findings are agreeing with the fact that alkali refining should improve the quality of groundnut oil by reducing the FFA. Evidence of improved the FFA was reported by Olaposi and Adunni (2010); the lower FFA in the oil samples indicates the stability of the products. The presence of FFA and other fatty materials in oil brings about the offensive odor and taste in oil on long storage (Aluyor *et al.*, 2009).

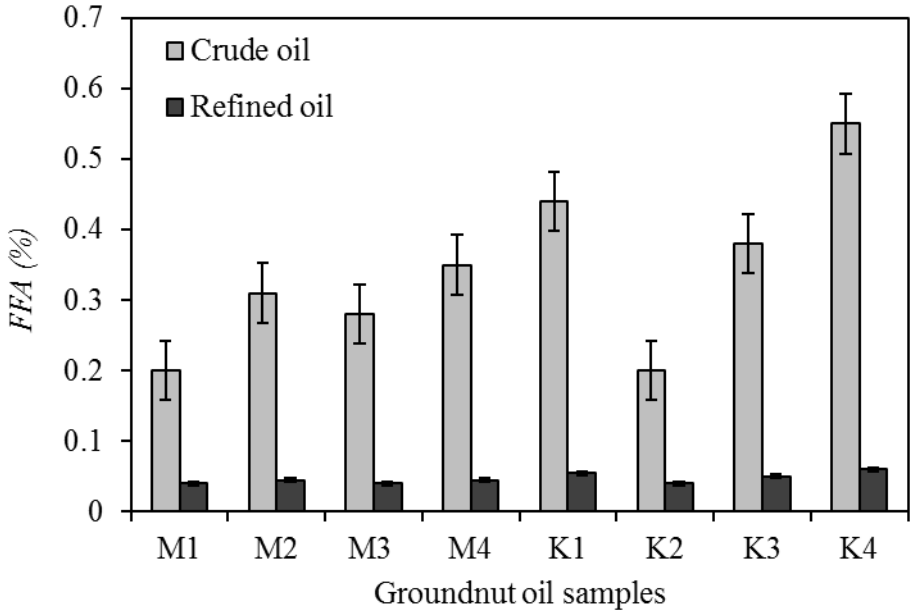

Figure 3: Free fatty acids of crude and refined groundnut oil from different expellers

CONCLUSIONS

Almost, all the samples of groundnut oil were affected by *A. flavus* and other fungi as well as contaminated with AFs. The addition of the caustic soda (alkali refining) to the groundnut oil has slight effect on the removal of AFs. The physicochemical properties of groundnut oil were investigated. The results revealed that alkali refining was improved significantly the quality of the groundnut oil by reducing the moisture content, acid value and free fatty acids percentage. However, the peroxide value was increased at acceptable levels. In short, alkali refining could restrain the speed of oil oxidation reaction thus extending the shelf-life of groundnut oil.

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تأثير عملية التكرير القلوي فى إزالة الأفلاتوكسينات وتقييم الجودة في زيت الفول السودانى

الملخـــص

في هـذا البحث تمت دراســـة تـأثير عمليـة التكرير القلوي على إزالـة الأفلاتوكســينات في زيـت الفول السـوداني. جُمعت عينات من زيت الفول السـوداني من عصـارات مختلفة من مدينتي ود مدنى والخرطوم في

السـودان. أُجريت دراسـة نسـبة الإصـابة بالفطر A. flavus والفطريات الأخرى في تلك العينات باسـتخدام الوسـط الغذائي (PDA). والتأكد من وجود الأفلاتوكسـينات عن طريق تقنية كروماتوجرافيا الطبقة الرقيقة (TLC) . أظهرت النتائج أن معظم عينات الزيت قد تأثرت بالفطر A. flavus والفطريات الأخرى وكذلك ملوثة بالأفلاتوكسـينات. أشـارت النتائج إلى أن عمليات التكرير القلوي لزيت الفول السـوداني لها تأثير ضـعيف على إزالة الأفلاتوكسـينات. علاوة على ذلك، كان هناك اختلاف كبير في الخصـائص الفيزيوكيميائية لزيت الفول السوداني بعد المعالجة عن طريق التكرير القلوي، وهذه تشمل محتوى الرطوبة، قيمة البيروكسيد، القيمة الحمضية وكذلك الأحماض الدهنية الحرة. ومن ناحية أخرى لم تكن هناك تغييرات كبيرة في مؤشر الانكسار قبل وبعد التكرير القلوي.