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Ultraviolet-LED irradiation effectively detoxified aflatoxin B1 in groundnut oils

Haibo Wang[a], Changbao Li[b,c,*], Ming Xin[b,c], Hock Eng Khoo[b,d], Zimei Mo[a], Songyu Zhou[a], Xiangdong Dai[a], Xiaocong Chen[a], Yiqing Nong[a], Juan-mei Zheng[a]

[a] Guangxi-ASEAN Food Inspection and Testing Center, Nanning 530021 China
[b] Agro-Food Science and Technology Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007 China
[c] Guangxi Key Laboratory of Fruits and Vegetables Storage-processing Technology, Nanning 530007 China
[d] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

*Corresponding author, e-mail: changbaoli@gxaas.net

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ABSTRACT: This study aimed to evaluate the quality of aflatoxin B1-detoxified unrefined groundnut oils. Detoxification of aflatoxin B1 (AFB1) in unrefined groundnut oils was performed by ultraviolet light-emitting diodes (UV-LED) irradiation. Ten samples of unrefined groundnut oils were subjected to UV-LED treatment before the measurements of AFB1 content, fatty acid compositions and chemical properties. The 20-min treatment reduced AFB1 content by 99%. Both acid and peroxide values of the oil samples showed little changes after the samples were treated with UV-LED irradiation. GC-MS results showed that 21 types of fatty acid were detected in the untreated oil samples. Minor changes in the levels of fatty acid were also observed after the 20-min treatment. The results proved that UV-LED irradiation effectively reduced AFB1 content in the unrefined groundnut oils and maintained the oil quality. It is a promising strategy for detoxification of AFB1 in groundnut oil.

KEYWORDS: *Aspergillus flavus*, fatty acid methyl esters, UV irradiation, aflatoxin, groundnut oils

INTRODUCTION

Aspergillus flavus and its metabolite aflatoxins (AFTs) pose health threats to humans, such as intoxication and cancers [1]. The fungus also contributes to huge economic losses in food, aquaculture and animal husbandry industries [2, 3]. Food crops are highly susceptible to AFT contamination during production, processing and transportation [4]. *Aspergillus flavus* contaminations in groundnut, soya bean and other food crops have been attributed to high environmental temperature and humidity.

Groundnut oil contains a range of unsaturated fatty acids and polyphenols. It is widely used as edible oil worldwide. The consumption of groundnut oil in China has increased from 2.81 million metric tonnes in 2016 to 3.08 million metric tonnes in 2019. It is considered an essential type of edible oil among the Chinese. In the rural areas of China, unrefined groundnut oil is highly used by the local communities for cooking food. The oil is prone to AFT contamination [5]. As reported in the literature, AFT contamination in unrefined edible oils available in Guangxi was a serious issue [6]. The report stated that unrefined edible oils, especially home-produced unrefined groundnut oils, were highly contaminated with aflatoxin B1 (AFB1). A recent study showed that unrefined groundnut oils produced locally in Guangdong Province of China were highly contaminated by AFB1 [7]. Therefore, the issue of AFT contamination in the unrefined groundnut oil is worrying. Agricultural scientists need to develop an effective and economical method for removing or detoxifying AFTs without affecting the quality of groundnut oil. On top of groundnut oil, AFT contamination in other edible oils is alarming. Hence, oil refining is a crucial step in detoxification of AFTs.

At present, there are three methods to detoxify AFTs in groundnut oil: physical, chemical and biotechnological [8–10]. Chemical treatment with chlorinating agents, oxidising agents or 75% methanol has effectively reduced AFTs content. However, the chemical residues pose as a major problem which limits its application in food industry. Application of ultraviolet (UV) radiation

in food industry replaces the use of dangerous chemicals. UV disinfection sources consist of primarily low-pressure and medium-pressure mercury lamps, which emit monochromatic and polychromatic lights, respectively. However, there are multiple drawbacks associated with the usage of UV light, which include large equipment size, high heat emission and high energy consumption, as well as the presence of mercury in foods [11].

Light-emitting diode (LED) has been used in highly efficient UV decontamination technology. UV-LED emits monochromatic light, which enables customised UV-LED detoxification system at specific wavelengths to be developed. The heat emission from LED is far lower than the traditional UV lamps. Thus, it is more suitable for application in food treatment, and it consumes less energy than the UV lamps [11]. UV irradiation is a popular method because of its high-energy. UV light induces complex photochemical reactions with AFTs. Literature has shown that UV irradiation is a cost-effective technique for detoxifying AFTs in biological samples [12, 13]. Previous studies also reported that UV irradiation reduced more than 90% of AFB1 in food products [12, 14, 15].

To date, there has been no report on the application of UV-LED irradiation technology for detoxification of AFTs in groundnut oil. Due to the seriousness of AFT contamination in unrefined groundnut oil and the health risk associated with dietary exposure to AFB1, there is a need to screen and evaluate AFB1 content and quality of the unrefined groundnut oil selling at the farmers' markets in Guangxi, China. In this study, we developed a novel method for detoxifying AFB1 in the unrefined groundnut oils using a patented AFT degradation machine. This machine has already been invented by applying UV-LED irradiation technology. Detoxification of AFB1 in unrefined groundnut oils was done according to the method developed, and the effects of UV-LED irradiation on qualities of the groundnut oils were determined.

MATERIALS AND METHODS

Chemicals, reagents and apparatus

AFB1 standard solution ($C_{17}H_{12}O_6$, CAS No: 1162-65-8, 2 μg/ml) and immunoaffinity column were obtained from Romer Labs China Ltd. (Beijing, China). BePure® fatty acid methyl ester (FAME) standards (purity ⩾99.0%) were purchased from Bestown (Beijing, China). Ultrapure water was prepared from a Milli-Q ultrapure water machine (Millipore Corporation, Milford, MA, USA). Reagents including methanol, *n*-heptane, acetonitrile and formic acid were of HPLC grade, and they were obtained from Merck Chemicals (Shanghai) Co., Ltd. (Shanghai, China). The others analytical grade reagents and chemicals including isooctane, n-hexane, diethyl ether, anhydrous sodium sulphate, sodium hydrogen sulphate and potassium hydroxide (KOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Samples preparation

Commercially available samples of unrefined groundnut oils were used in this AFTs detoxification study. The oil samples were randomly collected from ten different locations of farmers' markets in Guangxi during the summer season of 2019. Origins of the oils are shown in Table 1. Freshly collected oil samples were weighed and subjected to UV-LED irradiation. AFB1 stock solution was prepared by diluting the standard solution with methanol to 100 ng/ml (0.05 ml/ml in methanol). The stock solution was stored at −20 °C before HPLC analysis. A series of working standard solutions were prepared by diluting the stock solution with methanol at the following concentrations: 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 ng/ml. The working standard solutions of different concentrations were used for plotting standard calibration graph.

An analytical sample was prepared by mixing 1.0 g oil with 20 ml of methanol-water (70:30, v/v). The mixture was swirled for 20 min using a benchtop orbital shaker and then centrifuged for 10 min at 10 000 rpm. Then 4 ml of the supernatant was transferred to a new tube and mixed with 23 ml of PBS containing 1% TritonX-100. The sample solution was finally injected onto an immunoaffinity column at a flow rate of 1.0 ml/min; the column was rinsed with 10 ml of ultrapure water twice. The adsorbed components were eluted with 1 ml of methanol twice. The eluent was collected in a tube and evaporated to dryness under a stream of nitrogen at a temperature of 50 °C. The dried residue was dissolved with 1.0 ml of mobile phase and filtered through a 0.22 μm Millex® GP syringe filter (Merck Millipore Ltd., Carrigtohill, Ireland).

Treatment of unrefined groundnut oils with UV-LED irradiation

Unrefined groundnut oils with different concentrations of AFB1 were treated using an AFT degradation machine with a UV-LED detoxification system,

Table 1 AFB1 contents of unrefined groundnut oils treated at different irradiation times.

Sample	AFB1 content (μg/kg)						Origin of groundnut oils
	0 s	60 s	120 s	300 s	600 s	1200 s	
1	261.3 ± 1.12^{a}	10.9 ± 1.56^{b}	1.1 ± 2.45^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	Myanmar
2	147.9 ± 1.25^{a}	25.3 ± 1.54^{b}	6.8 ± 2.34^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	Hubei, China
3	1292.9 ± 0.53^{a}	627.4 ± 1.01^{b}	449.5 ± 1.21^{c}	273.7 ± 1.03^{d}	114.3 ± 1.11^{e}	18.2 ± 1.55^{f}	Henan, China
4	130.5 ± 1.01^{a}	12.7 ± 1.52^{b}	0.7 ± 2.64^{c}	0.4 ± 2.65^{c}	0.2 ± 2.34^{c}	0.1 ± 2.64^{c}	Vietnam
5	13.3 ± 2.33^{a}	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	Vietnam
6	653.5 ± 0.24^{a}	96.4 ± 1.24^{b}	20.0 ± 1.03^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	Henan, China
7	98.1 ± 1.34^{a}	7.2 ± 2.55^{b}	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	Vietnam
8	101.4 ± 1.13^{a}	7.8 ± 2.34^{b}	1.0 ± 1.62^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	Henan, China
9	389.1 ± 0.88^{a}	46.0 ± 1.56^{b}	5.1 ± 1.34^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	Hubei, China
10	70.2 ± 1.24^{a}	2.3 ± 2.55^{b}	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	South Africa

All data were presented as mean ± standard deviation of three replications. Different superscript lowercase letters denote a significant difference ($p < 0.05$). The oil samples were irradiated at the intensity of 3500 μW/cm^2.

and the patented equipment (Chinese patent number: ZL 2017 2 0215388.6) was specifically used for treating oil samples. The equipment is produced by the Guangxi Youqing Miyi Technology Co., Ltd. (Guangxi, China). In brief, 10 g oil sample was placed in a 25 ml glass colourimetric tube. The sample was exposed to UV-LED irradiation intensity of 3500 μW/cm^2 for 10–1200 s. UV-LED light intensity was set by adjusting the distance between colourimeter and UV lamp. The light intensity was measured using a photometer. The intensities of UV-LED light ($\lambda_{max} = 254$ nm) were set at 450, 750, 1250, 1550, 1850 and 3500 μW/cm^2 at distances of 12, 10, 7, 5, 2 and 0 cm, respectively; the irradiation time was set at 60 s. After the treatment, AFB1 content, fatty acid compositions, acid and peroxide values of all oil samples were determined. Triplicate analyses were performed on all samples.

HPLC analysis of AFB1

Changes in AFB1 content of the oil samples were determined by HPLC after irradiated using the UV-LED detoxification system. The filtrate was analysed by an HPLC system (Waters Alliance 2695 Separations Module, Milford, MA, USA) at a flow rate of 0.8 ml/min using an Agilent ZORBAX SB-C18 column (250 mm × 4.6 mm, 5 μm). Isocratic mobile phase used in separation of AFB1 was a mixture of methanol:acetonitrile:water (35:10:55 v/v/v). The column temperature was set at 35 °C, and the injection volume was 20 μl. Post-column photochemical derivatisation was performed with trifluoroacetic acid, and fluorescence detector was set at an excitation wavelength of 360 nm and an emission wavelength of 440 nm. A recovery test was done by spiking the untreated oil sample with a known amount of AFB1 standard. Each spiked sample was prepared by mixing 10 μg AFB1 standard and 1.0 g oil sample. Recovery of AFB1 in the oil sample was 90 ± 4% based on triplicate measurements.

GC-MS analysis of fatty acids

Fatty acid compositions of both irradiated and non-irradiated oil samples were determined using a Thermo Scientific TSQ™ 9000 triple quadrupole GC-MS/MS system (Shanghai, China). FAME stock solution was prepared in a 10-ml volumetric flask at a solution concentration of 5.0 mg/ml in n-heptane. The stock solution was stored in a −20 °C freezer before GC analysis. It was found to be remaining stable for over three months.

Analytical samples were prepared by mixing 100 mg oil and 10 ml of n-hexane in a 25-ml glass colourimeter tube. The mixture was agitated for 2 min, followed by the addition of 0.5 ml of KOH-methanol (0.5 M), and then ultrasonicated at a controlled temperature of 40 °C for 20 min. The mixture, after added with 5 ml of ultrapure water, was transferred to a new centrifuge tube and centrifuged at 10 000 rpm for 3 min at 25 °C. Supernatant obtained was transferred to a conical flask and treated with 2.0 g anhydrous sodium sulphate to remove excessive water. Before the GC-MS analysis, the supernatant was filtered through a 0.22 μm Millex® GP syringe filter. GC-MS analysis was performed in duplicate.

GC-MS analysis of fatty acids was performed based on the method provided by the Thermo Fisher Scientific Inc. [16]. Fatty acids in oil samples and FAME mixture were separated by using a TR-FAME (100 m × 0.25 mm × 0.2 μm) capillary column. Helium was used as a carrier gas with a flow rate of 1.0 ml/min; 1.0 μl injections were made in split mode, with a split ratio of 1:10. Injector temperature was also set at 250 °C. Analytes were separated

at a constant flow with the column temperature programmed at an initial 80 °C for 2 min at 30 °C/min, then 140 °C for 1 min, and finally, 240 °C at 2 °C/min for 5 min. Ion source temperature and interface temperature were set at 220 °C and 250 °C, respectively. The solvent delay time was 5.5 min. MS measurement was operated in electron impact (EI) mode and Q3 scan monitoring mode (m/z 40–450). Each peak was identified through comparison with the mass library of NIST 14. Quantitative analysis of fatty acids was done based on area normalisation method [17].

Acid and peroxide values of the unrefined groundnut oils

Acid and peroxide values are the critical quality attributes of oil samples. The determinations of acid and peroxide values of both irradiated and non-irradiated oil samples were done according to the methods described by Hussin et al [18], which were based on the AOCS Official Method Ca 5a-40 and Method Cd 8b-90, respectively [19, 20]. All oil samples were irradiated at an intensity of 3500 μW/cm^2 for 10, 30, 45, 60, 120, 300, 600 and 1200 s, except for the untreated oil sample. Acid and peroxide values of all oil samples were determined based on titration methods, and the values were expressed as mg/g and mmol/kg, respectively.

Statistical analysis

All data were presented in means of three replicates, except for the GC-MS data. The mean differences were statistically analysed using Minitab version 15.0 (Minitab Inc., PA, USA). Analysis of variance (ANOVA), coupled with the least significant difference was used for multiple comparisons. Significance was set at $p < 0.05$.

RESULTS

Effects of UV-LED irradiation time and intensity on AFB1 degradation in the unrefined groundnut oils

Working standard solution of AFB1 was injected in triplicate, and the response had high linearity, which showed a linear response range from 0.1 to 20 ng/ml ($R^2 = 0.9999$; $y = 2.399 \times 10^5 x - 3463$). The concentrations of AFB1 in ten samples of unrefined groundnut oil after the UV-LED treatment are presented in Table 1. The HPLC chromatograms of both sample and standard are shown in Fig. 1. ANOVA data showed that there were significant

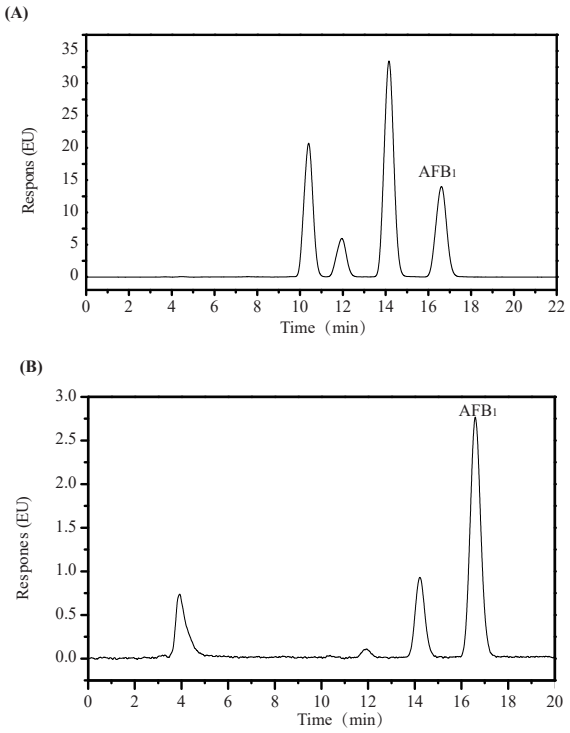

Fig. 1 HPLC chromatograms of (A) aflatoxin standard and (B) AFB1 in groundnut oil.

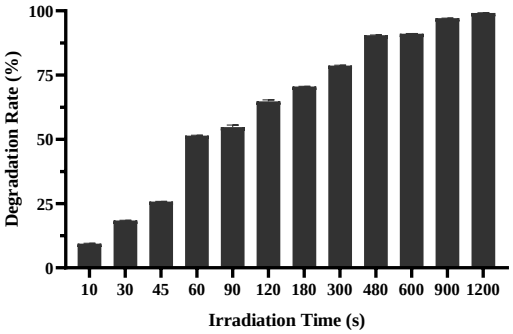

Fig. 2 Degradation rate of groundnut oil (sample 3) by applying irradiation intensity of 3500 μW/cm^2 at different irradiation times.

reductions in AFB1 content in all oil samples after the irradiations ($p < 0.05$).

As AFB1 absorbed UV, the irradiation activates AFB1 and increases its susceptibility to degradation. After being treated using the AFT degradation machine for different irradiation times at intensity of 3500 μW/cm^2, we found that the concentration of AFB1 in the oil samples reduced with increasing treatment duration. As AFB1 concentrations in nine

Table 2 Degradation effects of unrefined groundnut oils treated with different intensities ($\mu W/cm^2$) of UV-LED irradiation.

Sample	AFB1 content ($\mu g/kg$)						
	0	450	750	1250	1550	1850	3500
1	261.3 ± 0.85^{a}	118.0 ± 1.35^{b}	86.7 ± 1.22^{c}	75.1 ± 1.56^{d}	55.9 ± 1.54^{e}	29.9 ± 2.14^{f}	11.0 ± 2.55^{g}
2	147.9 ± 0.89^{a}	85.5 ± 1.04^{b}	79.5 ± 1.16^{c}	72.9 ± 1.11^{d}	59.9 ± 1.04^{e}	60.6 ± 1.25^{e}	26.9 ± 2.34^{f}
3	1292.9 ± 0.15^{a}	1210.0 ± 0.21^{b}	1101.0 ± 0.14^{c}	1092.0 ± 0.16^{c}	1056.0 ± 0.19^{d}	989.1 ± 0.20^{e}	632.8 ± 0.33^{f}
4	130.5 ± 1.05^{a}	63.3 ± 2.01^{b}	44.2 ± 2.14^{c}	37.7 ± 2.47^{c}	22.2 ± 2.56^{d}	9.2 ± 2.54^{e}	6.7 ± 2.65^{e}
5	13.2 ± 2.01^{a}	1.6 ± 2.55^{b}	1.2 ± 2.54^{b}	1.0 ± 2.31^{b}	0.8 ± 2.54^{b}	0.5 ± 2.55^{b}	0.0 ± 0.00^{c}
6	653.5 ± 0.51^{a}	362.8 ± 0.58^{b}	338.9 ± 0.57^{c}	269.2 ± 0.56^{d}	243.6 ± 0.68^{e}	139.7 ± 1.01^{f}	98.2 ± 1.58^{g}
7	98.1 ± 1.11^{a}	35.4 ± 1.25^{b}	30.4 ± 1.25^{b}	20.4 ± 1.34^{c}	15.2 ± 1.56^{d}	7.8 ± 2.14^{e}	7.4 ± 2.67^{e}
8	101.4 ± 1.02^{a}	42.6 ± 2.01^{b}	38.4 ± 2.47^{b}	24.6 ± 2.56^{c}	23.4 ± 2.54^{c}	13.0 ± 2.34^{d}	7.9 ± 2.72^{d}
9	389.1 ± 0.56^{a}	160.3 ± 0.68^{b}	142.5 ± 0.89^{c}	138.3 ± 0.98^{d}	76.7 ± 1.46^{e}	53.5 ± 1.89^{f}	46.7 ± 1.53^{g}
10	70.2 ± 1.68^{a}	23.8 ± 2.19^{b}	$17.3 \pm 2.75^{b,c}$	$14.0 \pm 2.63^{c,d}$	10.5 ± 2.71^{d}	$5.7 \pm 2.65^{d,e}$	2.5 ± 2.58^{e}

All data were presented as mean ± standard deviation of three replications. Different superscript lowercase letters denote a significant difference ($p < 0.05$). The oil samples were irradiated for 60 s.

samples of the unrefined groundnut oils were lower than 650 μg/kg, 2-min irradiation was enough to reduce AFT levels to 20 μg/kg or lower; no residue of AFB1 was found in the oil samples treated at 300 s irradiation. Due to the high concentration of AFB1 in sample 3 (up to 1292 μg/kg), its AFB1 concentration reduced to 273 μg/kg after the 300 s irradiation (Table 1). The decrement rate was about 80%. Hence, by applying a 20-min irradiation time, the degradation rate of AFB1 in sample 3 was up to 98% (Fig. 2).

The unrefined groundnut oils with different AFB1 concentrations were also treated with various irradiation intensities (450–3500 $\mu W/cm^2$) for 60 s. As showed in Table 2, the AFB1 degradation rate was proportional to irradiation intensity. Similarly, the AFB1 degradation rate of sample 3 was much lower than the other samples. The results also showed that the degradation rate was highly correlated with irradiation time and intensity, where the Pearson correlation coefficient r-values were 0.8–0.9.

Influence of UV-LED irradiation on quality of the unrefined groundnut oils

After the unrefined groundnut oils had been irradiated at different irradiation times and intensities using the UV-LED detoxification system, the temperature of the oils increased about 5 °C after a 20-min (1200 s) irradiation. When the irradiation times were below 5 min (300 s), no changes in the oil temperatures were observed (data not shown). As shown in Tables 3 and 4, there were no significant changes in acid and peroxide values, respectively ($p > 0.05$) after the oil samples were irradiated for 20 min by applying an irradiation intensity of 3500 $\mu W/cm^2$. The results also indicated that acid and peroxide values of all irradiated oil samples were not significantly different compared to the untreated samples ($p > 0.05$).

In this study, the main nutritional component of the unrefined groundnut oils was fatty acid; therefore, the number of fatty acids can be one of the useful criteria for measuring the quality of the oil samples besides acid and peroxide values. The oil samples were methylated, and fatty acid compositions of the oil samples were determined based on the in-house developed GC-MS method. Types of fatty acids of all oil samples were characterised by comparing retention times and molecular masses of the fatty acids between samples and standard. GC-MS chromatograms of FAME standards and oil sample are shown in Fig. 3.

The untreated groundnut oils had 21 types of fatty acid which included 67.51% unsaturated fatty acids and 32.49% saturated fatty acids. All-*cis*-9,12-octadecadienoic acid (linoleic acid), *cis*-9-octadecenoic acid (oleic acid) and *cis*-11-eicosenoic acid (gondoic acid) were the three highest unsaturated fatty acids in all samples, whereas hexadecanoic acid (palmitic acid), octadecanoic acid (stearic acid) and docosanoic acid (behenic acid) were the major saturated fatty acids. Considering that the response values of the fatty acid isomers are similar, the relative abundance value of each fatty acid can be determined by the area normalisation method, and the results are shown in Table 5. After being subjected to UV-LED irradiation, minor changes in the fatty acid compositions of all oil samples were observed. Hence, no changes in the percentages of *trans*-fatty acids in the oil samples were observed. Therefore, UV-LED irradiation did not alter fatty acid compositions of the unrefined groundnut oil. It might be because UV-LED irradia-

Table 3 Acid values of unrefined groundnut oils treated at different irradiation times.

Sample	Acid value (mg/g)								
	0 s	10 s	30 s	45 s	60 s	120 s	300 s	600 s	1200 s
1	1.84 ± 0.23	1.83 ± 0.21	1.82 ± 0.24	1.84 ± 0.34	1.84 ± 0.51	1.83 ± 0.45	1.84 ± 0.28	1.83 ± 0.42	1.84 ± 0.22
2	1.29 ± 0.32	1.28 ± 0.31	1.29 ± 0.29	1.28 ± 0.22	1.29 ± 0.19	1.29 ± 0.33	1.30 ± 0.22	1.28 ± 0.19	1.29 ± 0.33
3	12.12 ± 0.12	12.10 ± 0.15	12.08 ± 0.22	12.12 ± 0.21	12.12 ± 0.19	12.11 ± 0.28	12.10 ± 0.15	12.08 ± 0.13	12.11 ± 0.12
4	2.46 ± 0.24	2.45 ± 0.25	2.46 ± 0.24	2.45 ± 0.26	2.46 ± 0.23	2.45 ± 0.19	2.44 ± 0.18	2.45 ± 0.19	2.46 ± 0.21
5	0.39 ± 1.29	0.39 ± 1.05	0.38 ± 1.32	0.39 ± 1.12	0.38 ± 1.13	0.39 ± 1.32	0.39 ± 1.46	0.39 ± 1.24	0.38 ± 1.37
6	5.27 ± 0.26	5.25 ± 0.18	5.26 ± 0.14	5.25 ± 0.17	5.27 ± 0.19	5.28 ± 0.21	5.26 ± 0.22	5.27 ± 0.17	5.27 ± 0.14
7	2.14 ± 0.41	2.14 ± 0.33	2.13 ± 0.24	2.12 ± 0.29	2.13 ± 0.28	2.14 ± 0.31	2.13 ± 0.23	2.14 ± 0.24	2.13 ± 0.27
8	1.66 ± 0.34	1.66 ± 0.26	1.67 ± 0.24	1.66 ± 0.26	1.67 ± 0.25	1.66 ± 0.21	1.66 ± 0.23	1.67 ± 0.24	1.65 ± 0.27
9	3.78 ± 0.25	3.78 ± 0.19	3.77 ± 0.19	3.76 ± 0.22	3.78 ± 0.24	3.76 ± 0.19	3.77 ± 0.21	3.76 ± 0.19	3.78 ± 0.23
10	4.88 ± 0.27	4.86 ± 0.22	4.87 ± 0.21	4.86 ± 0.16	4.87 ± 0.17	4.87 ± 0.21	4.86 ± 0.23	4.85 ± 0.19	4.85 ± 0.25

All data were presented as mean ± standard deviation of three replications. The oil samples were irradiated at the intensity of 3500 $\mu W/cm^2$.

Table 4 Peroxide values of unrefined groundnut oils treated at different irradiation times.

Sample	Peroxide value (mmol/kg)								
	0 s	10 s	30 s	45 s	60 s	120 s	300 s	600 s	1200 s
1	0.35 ± 1.12	0.34 ± 1.13	0.35 ± 1.02	0.35 ± 1.10	0.34 ± 1.34	0.35 ± 1.15	0.34 ± 1.25	0.34 ± 1.32	0.34 ± 1.22
2	0.16 ± 1.32	0.15 ± 1.25	0.14 ± 1.24	0.15 ± 1.35	0.16 ± 1.28	0.14 ± 1.34	0.14 ± 1.41	0.14 ± 1.37	0.15 ± 1.28
3	0.11 ± 1.12	0.10 ± 1.05	0.11 ± 1.12	0.10 ± 1.25	0.11 ± 1.35	0.10 ± 1.41	0.10 ± 1.39	0.11 ± 1.38	0.11 ± 1.25
4	0.56 ± 0.55	0.55 ± 0.56	0.56 ± 0.45	0.55 ± 0.49	0.56 ± 0.52	0.55 ± 0.44	0.54 ± 0.53	0.53 ± 0.47	0.53 ± 0.25
5	1.33 ± 0.67	1.32 ± 0.56	1.33 ± 0.58	1.32 ± 0.46	1.33 ± 0.57	1.32 ± 0.48	1.33 ± 0.48	1.32 ± 0.51	1.30 ± 0.50
6	0.34 ± 0.82	0.34 ± 0.92	0.33 ± 0.82	0.34 ± 0.81	0.33 ± 0.86	0.33 ± 0.89	0.32 ± 0.91	0.33 ± 0.89	0.33 ± 0.89
7	0.60 ± 0.46	0.59 ± 0.49	0.60 ± 0.47	0.59 ± 0.48	0.59 ± 0.48	0.60 ± 0.46	0.59 ± 0.47	0.60 ± 0.43	0.59 ± 0.44
8	0.37 ± 0.44	0.38 ± 0.45	0.38 ± 0.39	0.39 ± 0.34	0.37 ± 0.24	0.39 ± 0.29	0.38 ± 0.31	0.39 ± 0.33	0.38 ± 0.34
9	0.22 ± 0.96	0.21 ± 0.92	0.21 ± 0.90	0.22 ± 0.94	0.21 ± 0.95	0.22 ± 0.92	0.21 ± 0.93	0.22 ± 0.94	0.21 ± 0.97
10	0.50 ± 0.46	0.49 ± 0.54	0.49 ± 0.52	0.49 ± 0.49	0.50 ± 0.52	0.48 ± 0.34	0.50 ± 0.58	0.48 ± 0.59	0.48 ± 0.59

All data were presented as mean ± standard deviation of three replications. The oil samples were irradiated at the intensity of 3500 $\mu W/cm^2$.

tion technology did not increase the oil temperature.

DISCUSSION

In this study, we investigated the reduction of AFB1 in the UV-LED-treated unrefined groundnut oils. UV-LED irradiation technology used in the AFT degradation machine could significantly reduce the concentrations of AFB1 in the unrefined groundnut oils ($p < 0.05$). Despite the reduction in levels of AFB1 in the unrefined groundnut oils, our findings showed that UV-LED irradiation did not significantly alter the fatty acid compositions, acid and peroxide values of the oils ($p > 0.05$). However, there was a slight increase in total saturated fatty acids (34.9%) in the treated oil samples after 20-min irradiation compared to the untreated oil sample (32.49%). It is because UV irradiation altered the double bonds of some unsaturated fatty acids, especially elaidic

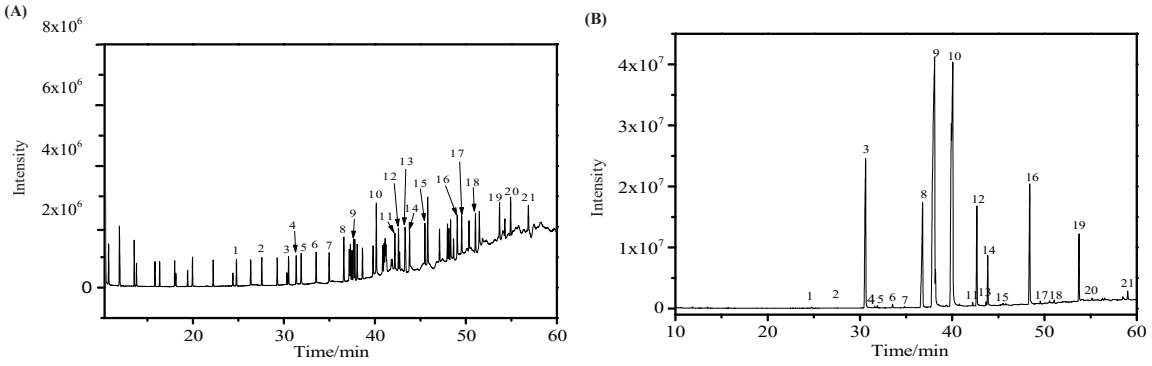

Fig. 3 GC-MS chromatograms of (A) FAME standards and (B) fatty acid methyl esters in groundnut oil. The fatty acids were identified as Peak 1–Peak 21 (Table 5).

Table 5 Fatty acid compositions of unrefined groundnut oils treated at different irradiation times.

Peak No.	Fatty acid			Retention time (min)	Concentration (%)					
					0 s	30 s	60 s	300 s	600 s	1200 s
1	Tetradecanoic acid, methyl ester	Myristic acid	C14:0	24.75	0.04	0.05	0.05	0.06	0.05	0.05
2	Pentadecanoic acid, methyl ester	Pentadecyclic acid	C15:0	27.54	0.01	0.02	0.01	0.01	0.01	0.02
3	Hexadecanoic acid, methyl ester	Palmitic acid	C16:0	30.61	10.09	11.3	11.29	11.34	11.25	11.27
4	*cis*-9-Hexadecenoic acid, methyl ester	Palmitoleic acid	C16:1,n-7	31.60	0.05	0.08	0.07	0.09	0.07	0.07
5	*trans*-9-Hexadecenoic acid, methyl ester	*trans*-Palmitoleic acid	C16:1,n-7	31.88	0.07	0.10	0.10	0.10	0.09	0.10
6	Heptadecanoic acid, methyl ester	Margaric acid	C17:0	33.53	0.12	0.18	0.16	0.18	0.17	0.17
7	*cis*-10-Heptadecenoic acid, methyl ester	Heptadecenoic acid	C17:1,n-7	34.82	0.04	0.06	0.06	0.06	0.06	0.06
8	Octadecanoic acid, methyl ester	Stearic acid	C18:0	36.79	7.95	9.59	9.47	9.54	9.44	9.43
9	*cis*-9-Octadecenoic acid, methyl ester	Oleic acid	C18:1,n-9	38.09	35.35	34.3	34.44	34.3	34.43	34.4
10	All-*cis*-9,12-Octadecadienoic acid, methyl ester	Linoleic acid	C18:2,n-6	40.06	29.68	27.8	27.85	27.69	28.01	28.1
11	All-*cis*-9,12,15-Octadecatrienoic acid, methyl ester	α-Linolenic acid	C18:3,n-3	42.22	0.13	0.13	0.13	0.14	0.13	0.13
12	Eicosenoic acid, methyl ester	Arachidic acid	C20:0	42.67	4.58	4.54	4.54	4.61	4.51	4.45
13	*cis*-5-Eicosenoic acid, methyl ester	5-Eicosenoic acid	C20:1,n-15	43.67	0.14	0.14	0.14	0.15	0.14	0.14
14	*cis*-11-Eicosenoic acid, methyl ester	Gondoic acid	C20:1,n-9	43.85	1.95	1.94	1.91	1.88	1.90	1.90
15	Heneicosanoic acid, methyl ester	Heneicosylic acid	C21:0	45.50	0.06	0.07	0.06	0.06	0.06	0.06
16	Docosanoic acid, methyl ester	Behenic acid	C22:0	48.40	6.27	6.33	6.33	6.34	6.31	6.24
17	*trans*-13-Docosenoic acid, methyl ester	Brassidic acid	C22:1,n-9	49.54	0.10	0.11	0.10	0.10	0.10	0.10
18	Tricosanoic acid, methyl ester	Tricosylic acid	C23:0	51.05	0.11	0.11	0.11	0.11	0.11	0.11
19	Tetracosanoic acid, methyl ester	Lignoceric acid	C24:0	53.74	2.83	2.78	2.77	2.74	2.74	2.68
20	Pentacosanoic acid, methyl ester	Pentacosylic acid	C25:0	56.28	0.06	0.07	0.06	0.11	0.06	0.07
21	Hexacosanoic acid, methyl ester	Cerotic acid	C26:0	59.02	0.37	0.38	0.35	0.39	0.36	0.35

All data were presented as means of two replicates. The oil samples were irradiated at the intensity of 3500 $\mu W/cm^2$.

acid and petroselaidic acid [21]. Elaidic acid is the stereoisomer of oleic acid, where oleic acid is the major monounsaturated fatty acid in groundnut oil. These fatty acids (oleic, elaidic and petroselaidic acids) share the same molecular weight of 282.46 g/mol.

On the other hand, the unrefined groundnut oils could be adulterated with animal fat. The GC-MS data show that pentadecanoic (C15:0), heneicosanoic (C21:0), tricosanoic (C23:0) and pentacosanoic (C25:0) acids were detected in the oil samples. These saturated fatty acids are not commonly found in vegetable oils. Therefore, future studies need to focus on the adulteration of groundnut oil with animal fats on top of the AFB1 detoxification. Although the quality of the unrefined groundnut oil is maintained, degradation products of AFB1 are still needed to be identified, especially the *in vivo* toxicity and mutagenicity of the components. A previous study identified the degradation products of AFB1 in groundnut oil treated with UV irradiation as compounds P1 ($C_{18}H_{33}N_3O_3$) and P2 ($C_{12}H_{22}N_2O_2$) [22]. These compounds have lower toxicity levels than AFB1 ($C_{17}H_{12}O_6$).

AFT contamination in food occurs mainly due to high temperature and humid conditions. The levels of AFB1 detected in groundnut extracts ranged from 0.559–1.550 μg/g extract [23]. It is far higher than the levels determined in other nuts and grains [24]. In a recent study, two biodegraded products of AFB1 obtained from treatment with culture supernatant of *Cladosporium uredinicola* were structurally identified as $C_{19}H_{18}O_{10}$ and $C_{18}H_{14}O_7$; these compounds were reported to be less toxic than AFB1 based on quantitative structure-activity relationship and cytotoxicity experiment [25]. Besides the concern for AFT contamination and its impacts on consumer health, it is necessary to take preventive and proper measures to reduce the levels of contamination below the regulatory limits.

Literature has shown that gamma irradiation inhibited the growth of aflatoxigenic moulds on corn seeds, thus reduced AFB1 formation [26]. Gamma irradiation also effectively reduced AFT accumulation in black and white peppers regardless of the moisture content of peppers [27]. In contrast, Di Stefano et al [28] reported that gamma irradiation reduced tocopherol content in almond at increasing irradiation doses. In addition to gamma irradiation, temperature-controlled pulsed light treatment has been shown to reduce AFT levels in treated groundnut oils. However, there is a limitation in using

this method due to the high temperature generated during the irradiation. The results showed that the oil's temperature increased from 26 °C to 220 °C during the 10-min temperature-controlled pulsed light treatment [29]. The high temperature reduced the oil quality by elevating the peroxide value, acid value and percentage of free fatty acid in the oil after 400 s irradiation. In contrast, inactivation of AFB1 in groundnuts using pulse light for 300 s at 5 cm distance caused a burnt surface without affecting the groundnut's quality [30].

Advancement in UV technology replaces the traditional ways to detoxify AFTs in nuts and grains. UV irradiation has been discovered for its use in the detoxification of AFB1 in nuts due to their photosensitivity [31]. In the past, UV-C has been applied in the removal of AFT in nut samples. Basaran reported that a single dose (6 h irradiation) of UV-C treatment sufficiently reduced almost 25% of AFB1 in the treated hazelnut without affecting its sensory properties [32]. The review by Diao et al [12] showed that UV irradiation with different irradiation conditions effectively reduced more than 86% of AFT in groundnut oil. Moreover, UV irradiation has been reported to reduce polyphenol oxidase activity [33]. Thus, it helps to protect polyphenols in the oil sample. The findings of this study indicate that UV-LED irradiation effectively reduces the levels of AFB1 in the unrefined groundnut oils. The application of UV-LED irradiation technology in detoxification of AFB1 in groundnut oil is a more promising way compared with the use of other methods. It is also an economical way for the food processing industry to reduce AFT contamination in oils and other food products. However, UV detoxification efficiency is still the focal point. Development of advanced equipment will be necessary for food protection in the future.

CONCLUSION

Detoxification of AFTs by UV-LED irradiation technology is an innovative way of maintaining the quality of groundnut oils. The UV-LED detoxification system is considered being a green technology, where it generates low heat. Thus, it does not destroy unsaturated fatty acids of groundnut oils. UV-LED irradiation duration and intensity were the two factors that affect AFB1 content in the unrefined groundnut oils. The degradation of AFB1 was dependent on irradiation time and intensity. UV-LED irradiation also did not alter acid and peroxide values and fatty acid compositions of the treated oil samples compared with the untreated.

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