

MONITORING TRANSMISSION OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) LEVELS THROUGH PROCESSED ARACHIS HYPOGEA SHELLS TO SEEDS

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Abstract: Arachis Hypogea, a common delicacy consumed either boiled or roasted in most parts of Nigeria was assessed to determine the level of transmission of Polycyclic Aromatic Hydrocarbons (PAHs) through the shells to the seeds following boiling and roasting. Samples collected were extracted with a mixture of hexane (75%) and dichloromethane (25%) then analyzed with gas chromatography coupled to flame ionization detector. The results revealed that the total PAH concentrations ($\mu\text{g/kg}$) in the groundnut shell samples were un-boiled (1.1816) and boiled (1.0924) while the concentrations in the seeds were 0.6303, 0.5868 respectively. For the roasted groundnut; Total PAH concentrations in the non-roasted and roasted shells were 0.5164, 1.0384 while the total PAH concentrations in the seeds were 0.5292 and 0.6144 respectively. The % PAH Contamination Level (PCL) revealed that processing by boiling contaminated the shell with Fluoranthene (58 %), Benzo[b]Fluoranthene (31%) and Benzo[a]Pyrene (24%) while only acenaphthylene contaminated the seed by this process. On the other hand, % PAH contamination levels for

roasted groundnut shells was in the range of 0-92 % with Fluorene, Chrysene, Benzo[b]Fluoranthene, Indeno[1,2,3-cd]Pyrene and Benzo[g,h,i]Perylene not contaminating it by the process while Fluoranthene (92%) was the most contaminated PAH. Similarly, the seed was contaminated in the range of 0-29% with only acenaphthylene not contaminating it by the process. However, the level of contamination were lower than observed for the shell which could suggest that the shells shielded the seeds from much contamination by the PAHs while those that contaminated the seeds could have been by absorption through the pores. Furthermore, the PAH transfer Potential (PTP) determined from the study indicated there were varying degrees of potential PAH transport from the shells to the seeds at values >0 . The study recommends that boiled groundnut seed could have less potential for cancer causing than the roasted groundnut seed based on the level of contamination. Sensitization on the likely effect of consuming these processed foods should be promoted.

Keywords: Aromatic, Boiled, Monitoring, PAH, Roasted, Transmission

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1.0 Introduction

Groundnuts otherwise known as peanuts (*Arachis Hypogaea*) is an oil seed legume that is a staple nut commonly consumed in most parts of the world and having many nutritional and medicinal benefits (Abubakar & Ahmad, 2022)[1]. They are usually consumed raw, boiled or roasted. Boiled and roasted groundnuts are regarded as processed because they required set of methods and techniques needed to transform the raw forms to other forms that could be consumed by humans or animals (Mada *et al.*, 2012)[2].

The processing activity can contribute Polycyclic Aromatic Hydrocarbons (PAHs) to the processed groundnut. Generally, PAHs have been attributed to contamination of the environment and during food processing (Palade *et al.*, 2023) [3]. Furthermore, Smoking, frying and grilling have been reported to contribute PAHs than the raw or cold forms (Zhao *et al.*, 2023) [4]. Polycyclic Aromatic Hydrocarbons are a group of organic pollutants which have been found to result from the incomplete combustion of materials in the environment such as garbage, Petroleum products, meat, food among others (Lau *et al.*, 2010)[5]. PAHs such as Benzo [a] Pyrene, Benz[a]anthracene, benzo[b]Fluoranthene and Chrysene usually described as PAH4 among other PAHs have been known to show carcinogenic Potentials (Oko & Okoye, 2017)[6]. These class of organic pollutants are usually lipophilic hence they bioaccumulate in adipose tissues which may be the reason for their contamination of fatty foods (Ingenbleek *et al.*, 2019)[7]. Groundnuts are actually oil containing foods.

The idea of Transfer factor has been applied in monitoring heavy metal accumulation in plants from soils (Sharafi *et al.*, 2024) [8]. However, to the best of our knowledge, studies monitoring PAH transfer from one part of a plant or fruit to another have not been reported especially in Nigeria which is the reason for embarking on this study to assess the extent of potential transfer of the pollutants from the shell to the seed during the boiling and roasting process.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and preparation

Fresh groundnuts were purchased from three vendors at the old market in Wukari town of Wukari Local Government Area, Taraba state. The samples as purchased were aggregated together in a bowl to ensure homogeneous mixing.

The samples were prepared by dividing the fresh samples into two portions; one fresh and the other to be dried. The fresh sample was further divided into two portions. One portion was put into a cooking pot and heat applied from a hot plate for a period of 15-20 mins then the hot plate was put off and it was allowed to cool. Both the boiled and fresh groundnuts were dehulled separately and air dried for about two days.

The dried portion of the groundnut was divided into two parts, one part was placed in a frying pan and roasted on a hot plate for about 15-20 minutes. Both portions of dried and roasted groundnuts were dehulled. The shells and seeds of the fresh, dried, boiled and roasted groundnut were pulverized using a clean mortar and pestle separately then packaged in a black polythene bags prior to laboratory analysis.

2.2 PAH EXTRACTION

The PAHs were extracted using the modified method 3500C [9]. Exactly 2g of the sample was weighed on an analytical balance and transferred into a beaker then 10 ml of a mixture of redistilled hexane and dichloromethane in a ratio of 3:1 was added. The beaker and content were placed in a sonicator to extract the hydrocarbon for two hours. The Organic layer was filtered into a 250 ml capacity borosilicate beaker.

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2.3 CLEAN UP AND ANALYSIS

The concentrated oil was separated into the aliphatic and hydrocarbon profiles by packing the column with activated Alumina. The activated Alumina packed in the column was properly cleaned with redistilled hexane to remove aliphatic profiles into a pre-cleaned 20 ml capacity glass container. The Polycyclic aromatic hydrocarbon was then recovered by allowing the mixture of hexane and dichloromethane in the ratio 3 to 1 to run through the Alumina packed column and finally removed the most polar PAH by allowing the redistilled dichloromethane to run through the packed column and collected into the pre-cleaned borosilicate beaker. The mixture was then concentrated to 1 ml by nitrogen gas before gas chromatographic analysis. 1 ml of the concentrated extract was injected into the gas chromatograph column through the injection port and the components detected with flame ionization detector.

2.4 Gas chromatographic conditions/parameters

Model: HP6890; Column: HP-1; Column length/Column internal diameter/Column film: 30m, 0.25 µm, 0.25 µm; Injection temperature: 250°C; Detector temperature: 320°C; Detector: Flame Ionization detector; Initial temperature: 60°C for 5 minutes; First rate 15°C/min for 14 minutes and maintained for 3 minutes; Second rate: 10°C/minute for 5 minutes and maintained for 4 minutes; Mobile phase or carrier: Nitrogen; Nitrogen column pressure: 30 psi; Hydrogen pressure: 28 Psi; Compressed air pressure: 32 psi

2.5 Quality control check

The gas chromatograph was calibrated using standard solutions of the pure standard mix and the PAHs in samples identified by comparing them with retention times of the peaks in the pure standard mix. Furthermore, blanks were used after analysis of each two sample to check if laboratory sources contaminated the samples. The blanks were prepared by substituting redistilled n-hexane in the place of the sample composite and performing the usual extraction procedure as earlier described.

2.6 Determination of % PAH contamination Level from boiling and roasting process

The study adopted the view of Oko and Okoye, 2021[10] with modifications to determine the % PCL due to the boiling and roasting. Consequently,

$$\% \text{ PAH Contamination Level from boiling} = \frac{C_{bs}(\text{boiled}) - C_{ubs}(\text{unboiled})}{C_{bs}(\text{boiled})} \text{ ----- (1)}$$

C_{bs} = Concentration of PAH in samples after boiling

C_{ubs} = Concentration PAH in samples before boiling

And for the processing due to roasting

$$\% \text{ PAH Contamination Level from Roasting} = \frac{C_{Rs}(\text{Roasted}) - C_{uRs}(\text{non roasted})}{C_{Rs}(\text{Roasted})} \text{ ----- (2)}$$

C_{Rs} = Concentration of PAH in samples after Roasting

C_{uRs} = Concentration PAH in samples before Roasting

% PAH Contamination Level from smoking	Interpretation
70-100	Very high Contamination
50-69	High Contamination
21-49	Fair Contamination
1-20	Low Contamination
0	No Contamination

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Source: Oko and Okoye, 2021[10]

The study also introduced the concept of PAH Transfer potential (PTP) to assess the extent of Potential transmission of PAH from the shell of the boiled and Roasted samples to the seeds. This was carried out using the following equation;

$$\text{PAH Transfer Potential (PTP)} = \frac{C_{\text{PAHseed}}(\mu\text{g/kg})}{C_{\text{PAHShell}}(\mu\text{g/kg})} \text{-----} (3)$$

C_{PAHseed} = Concentration of PAH in seed of the boiled or roasted groundnut

C_{PAHShell} = Concentration of PAH in shell of the boiled or roasted groundnut

Key:

PTP < 1 = Indicates greater affinity of PAH to the Shell than the seed

PTP > 1 = Indicates less affinity of PAH to the Shell than the seed.

3.0 Results and discussion

The Concentrations of the 16 USEPA listed PAH in the fresh and boiled groundnut shell and seed are presented in Table 1. The results show that the total PAH concentrations in the shells and seeds of the fresh or un-boiled groundnuts were 1.1816 and 0.6303 $\mu\text{g/kg}$ respectively which were slightly higher than the total concentrations in the boiled shells and seeds (1.0924 and 0.5868 $\mu\text{g/kg}$ respectively) which implied that the heat generated from the boiling process may have brought about degradation. High temperatures can cause degradation of PAHs. Furthermore, of the PAH4 detected, Benzo[a]anthracene and Benzo[b]Fluoranthene had higher concentrations in the un-boiled seeds than the shells which indicated that these PAHs were contained from the environment before the boiling process. Chrysene and Benzo[a]Pyrene however contained more of the PAHs in the un-boiled shells than the seeds.

The results of PAH concentrations in the non- roasted and roasted shell and seeds of *Arachis Hypogea* are presented in Table 2. The results show that the roasting process increased the level of PAHs since the non-roasted shell and seed had concentrations of 0.5092 and 0.5292 $\mu\text{g/kg}$ respectively which were lower than the total concentrations in the roasted shell and seed with concentrations 1.0384 and 0.6144 $\mu\text{g/kg}$ respectively. The results further revealed that the roasting process contributed more PAHs to the shell than the seed.

The % PCL due to the processing methods are presented in Table 3. The results show that only fluoranthene (58%), Benzo[b]Fluoranthene (31%) and Benzo[a]Pyrene (24%) contaminated the shell from the boiling process. All the other PAHs contaminated the shell from the environment. The high level of contamination of the shell by fluoranthene from the boiling process could be due to its lower molecular weight compared to Benzo[b]Fluoranthene and Benzo[a]Pyrene.

For the Seeds, only acenaphthylene (15%) contaminated it from the boiling process. The low contamination of the seed despite its low molecular weight maybe attributed to shielding of the seed by the shell during the boiling process.

In the roasting process, most of the PAH contaminated the shells except Fluorene, chrysene, benzo[b]Fluoranthene, Indeno[1,2,3-cd]Pyrene and benzo[g,h,i]Perylene. Acenaphthylene (78%), Acenaphthene (75%), Fluoranthene (92%), Pyrene (79%) and dibenz(a,h)anthracene (74%) had very high contaminations while Phenanthrene (47%), fair contaminations and Naphthalene (36%), Anthracene (2%), benzo[k]Fluoranthene, Benzo[a]Pyrene exhibited low contamination of the groundnut shells.

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For the seeds, only Acenaphthylene did not contaminate the seed through the roasting process. The contamination Levels of some of the PAHs in the range of 5-20% exhibited a low contamination of the seed by the roasting process while dibenz[a,h]anthracene (28%) and indeno[1,2,3-cd]pyrene (29%) fairly contaminated the seeds. None of the PAHs were highly contaminated. The results obtained in the present study were comparable to a study carried out by Oko and Okoye, 2021[10] were % PAH contamination levels from smoke to roasted beef were in the ranges of 0-96% with acenaphthylene (72%) and Benzo[a]anthracene (89%) very highly contaminated the beef just as seen in the present study that the roasted groundnut shell was also highly contaminated. Phenanthrene and Benzo[a]pyrene which highly contaminated the beef in that study exhibited low contamination of the shell in the present study.

The % PCL from boiling clearly indicated that boiling did contribute all the PAHs to the shell and seeds but rather for the roasting process, PAHs in the Shell and seed were contributed to the shells and seed from the environment prior to the boiling process. Roasting however contributed most of the PAHs to the Shell than the seeds from the process.

The result of PAH transfer Potential (PTP) is presented in Table 4. The order of potential transfer due to the boiling process in decreasing order was Benzo[a]Anthracene (24.00) > Acenaphthylene (10.84) > Fluorene (10.40) > Benzo[b]Fluoranthene (4.44) > Acenaphthene (4.03) > Benzo[k]Fluoranthene (2.05) > Naphthalene (2.03) > Phenanthrene (1.28) > Benzo[g,h,i]Perylene (0.95) > Dibenz[a,h]anthracene (0.88) > Indeno[1,2,3-cd]Pyrene (0.60) > Chrysene (0.56) > Benzo[a]pyrene (0.41) > Anthracene (0.33) > Pyrene (0.29) > Fluoranthene (0.12).

The results show that the boiling process did not contribute majority of the PAHs to the shell and seed of the groundnut unlike the roasting process which contributed varying degrees of the PAHs. However, there were varying degrees of transfer which may have resulted from the heat degrading the PAHs and their passage through the shell pores to the seeds.

The process of roasting of the groundnuts when compared to boiling did not indicate greater potential for transfer of the PAHs from the shell to the seeds. The order of transfer potential include; Benzo[a]Anthracene (11.00) > Acenaphthylene (2.72) > Benzo[b]Fluoranthene (2.03) > Acenaphthene (1.39) > Chrysene (1.35) > Naphthalene (1.08) > Fluorene (1.07) > Benzo[k]Fluoranthene (0.81) > Indeno[1,2,3-cd]Pyrene (0.78) > Anthracene (0.69) > Benzo[a]Pyrene (0.68) > Dibenz[a,h]anthracene (0.61) > Fluoranthene (0.40) > Benzo[g,h,i]Perylene (0.33) > Pyrene (0.13) > Phenanthrene (0.10).

In both the boiling and roasting processes, Benzo[a]Pyrene which has been implicated majorly in carcinogenicity indicated a higher affinity to the shell than the seed implying less transfer from the shell to the seed. Furthermore, in both the boiling and roasting process Benzo[a]anthracene and Acenaphthylene respectively indicated the highest affinity to the seeds than the shells. The reason for this is not clear however it may be due to their structure and the size of the pores on the groundnut shells through which they passed onto the seeds.

4.0 Conclusion

The study determined Polycyclic Aromatic Hydrocarbon levels in boiled and roasted groundnuts (*Arachis Hypogaea*) shells and seeds in the bid to assess the extent of transmission of the sixteen priority listed PAHs from the shells to the seeds. The % PAH contamination level from boiling to the shell was reported only for Fluoranthene (58%), Benzo[b]Fluoranthene (31%) and Benzo[a]Pyrene (24%) while only acenaphthylene

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(15%) contaminated the seed. Similarly, for the roasted shell, apart from Fluoranthene, Chrysene, Benzo[b]Fluoranthene, Indeno[1,2,3-cd]Pyrene and Benzo[g,h,i]Perylene all other sixteen priority listed PAHs contaminated the shell in varying degrees. Only acenaphthylene did not contaminate the seed from the roasting process, all the others contaminated the seed in the range of 5-29%.The study also established evidence of transfer of PAH from the shell to the seed probably via absorption through the shell pores. The boiling process indicated higher potential for transfer from the shell to the seed for most of the PAHs compared to the roasting process. The Benzo[a]pyrene concentrations were within the Food standard Agency limits of 2µg/kg (Food standard agency, 2012). However, the study recommends that boiled groundnut seed consumption may have less potential for cancer causing as a result of contamination of PAHs from the process.

Table 1: PAH concentrations in fresh/ boiled groundnut shells and seeds

S/N	PAHS	CONCENTRATIONS (µg/kg)			
		FRESH SHELL	BOILED SHELL	FRESH SEED	BOILED SEED
1.				0.2849	
	Naphthalene	0.1962	0.1372		0.2785
2.		0.0899	0.0025	0.0229	0.0271
	Acenaphtylene				
3.		0.0167	0.0038	0.0172	0.0153
	Acenapthene				
4.		0.0064	0.0057	0.0706	0.0593
	Fluorene				
5.		0.0155	0.0111	0.0159	0.0142
	Phenanthrene				
6.		0.1425	0.0761	0.0325	0.0251
	Anthracene				
7.		0.2681	0.6376	0.0817	0.0742
	Fluoranthene				
8.		0.3316	0.1831	0.0582	0.0526
	Pyrene				
9.		0.0012	0.0006	0.0158	0.0144
	Benzo[a]Anthracene				
10.		0.0033	0.0048	0.0031	0.0027
	Chrysene				
11.		0.0017	0.0009	0.0045	0.004
	Benzo[b]Fluoranthene				
12.		0.0055	0.002	0.0046	0.0041
	Benzo[k]Fluoranthene				
13.	Benzo[a]Pyrene	0.0136	0.0179	0.0086	0.0073

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14.	Indeno[1,2,3-cd]Pyrene	0.0006	0.0005	0.0004	0.0003
15.	Dibenz[a,h]Anthracene	0.0802	0.0067	0.0072	0.0059
16.	Benzo[g,h,i]Perylene	0.0086	0.0019	0.0022	0.0018
	TOTAL	1.1816	1.0924	0.6303	0.5868

Table 2: PAH Concentrations in unroasted/roasted groundnut shell and seed

S/N	PAHS	CONCENTRATIONS (µg/kg)			
		UNROASTED SHELL	NON-ROASTED SEED	ROASTED SHELL	ROASTED SEED
1	Naphthalene	0.1640	0.2508	0.2582	0.2782
2	Acenaphthylene	0.0014	0.0191	0.0064	0.0174
3	Acenaphthene	0.0067	0.0306	0.0271	0.0376
4	Fluorene	0.0711	0.0449	0.0502	0.0538
5	Phenanthrene	0.0514	0.0078	0.0979	0.0096
6	Anthracene	0.0889	0.0448	0.0909	0.0623
7	Fluoranthene	0.0118	0.0487	0.1476	0.0586
8	Pyrene	0.0635	0.0369	0.2959	0.0389
9	Benzo[a]Anthracene	0.0005	0.0101	0.0011	0.0121
10	Chrysene	0.0036	0.0019	0.0017	0.0023
11	Benzo[b]Fluoranthene	0.0042	0.0048	0.0029	0.0059
12	Benzo[k]Fluoranthene	0.0047	0.0028	0.0043	0.0035

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13				0.0311	0.0212
	Benzo[a]Pyrene	0.0250	0.0162		
14	Indeno[1,2,3-cd]Pyrene	0.0015	0.0005	0.0009	0.0007
15	Dibenz[a,h]Anthracene	0.0045	0.0081	0.0176	0.0108
16	Benzo[g,h,i]Perylene	0.0064	0.0012	0.0046	0.0015
					0.6144
				1.0384	
	TOTAL	0.5092	0.5292		

Table 3: % PAH Contamination Levels from processing to groundnut shell and seed

S/NO	PAHs	PAH Contamination Levels (%)			
		BOILED SHELL	BOILED SEED	ROASTED SHELL	ROASTED SEED
1.	Naphthalene	0	0	36	10
2.	Acenaphthylene	0	15	78	0
3.	Acenaphthene	0	0	75	19
4.	Fluorene	0	0	0	17
5.	Phenanthrene	0	0	47	19
6.	Anthracene	0	0	2	28
7.	Fluoranthene	58	0	92	17
8.	Pyrene	0	0	79	5
9.	Benzo[a]Anthracene	0	0	55	17
10.	Chrysene	0	0	0	17
11.	Benzo[b]Fluoranthene	31	0	0	19
12.	Benzo[k]Fluoranthene	0	0	17	20
13.	Benzo[a]Pyrene	24	0	20	24
14.	Indeno[1,2,3-cd]Pyrene	0	0	0	29
15.	Dibenz[a,h]Anthracene	0	0	74	23
16.	Benzo[g,h,i]Perylene	0	0	0	20

Table 4: PAH Transfer Potential from shell to seeds of boiled and Roasted groundnuts

S/No	PAH	TF(Boiled)	TF(Roasted)
1.	Naphthalene	2.03	1.08
2	Acenaphthylene	10.84	2.72
3.	Acenaphthene	4.03	1.39
4.	Fluorene	10.40	1.07
5.	Phenanthrene	1.28	0.10
6.	Anthracene	0.33	0.69

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7.	Fluoranthene	0.12	0.40
8.	Pyrene	0.29	0.13
9.	Benzo[a]Anthracene	24.00	11.00
10.	Chrysene	0.56	1.35
11.	Benzo[b]Fluoranthene	4.44	2.03
12.	Benzo[k]Fluoranthene	2.05	0.81
13.	Benzo[a]Pyrene	0.41	0.68
14.	Indeno[1,2,3-cd]Pyrene	0.60	0.78
15.	Dibenz[a,h]Anthracene	0.88	0.61
16.	Benzo[g,h,i]Perylene	0.95	0.33

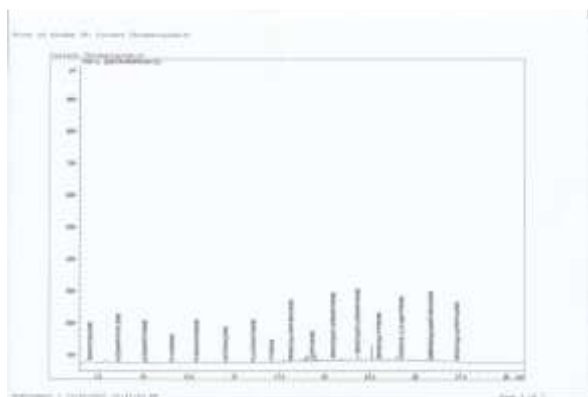


Fig 1: Chromatogram for unroasted groundnut shell

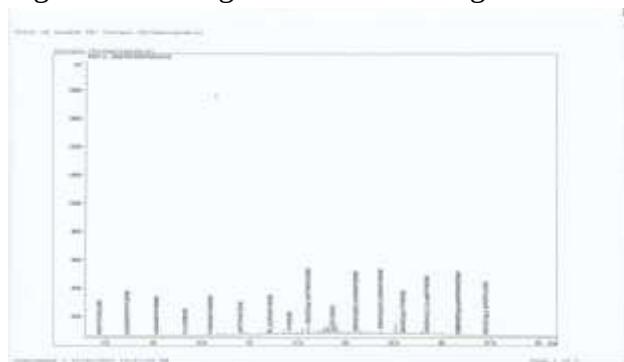
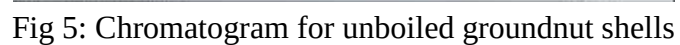
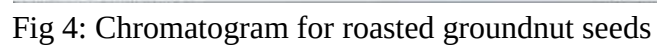
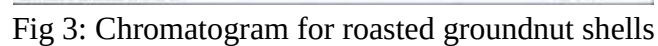


Fig 2: Chromatogram for unroasted groundnut seeds



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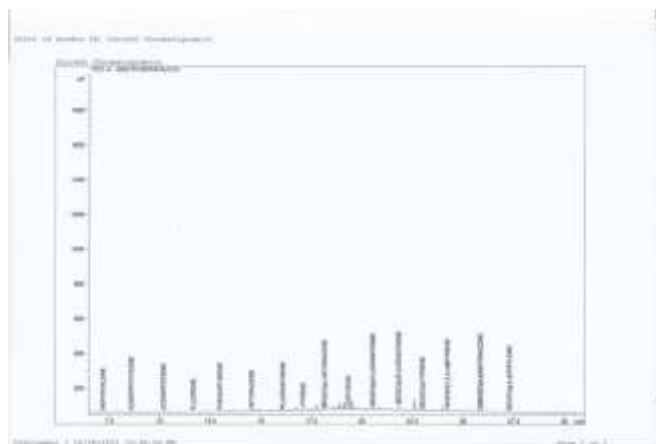


Fig 6: Chromatogram for unboiled groundnut seeds

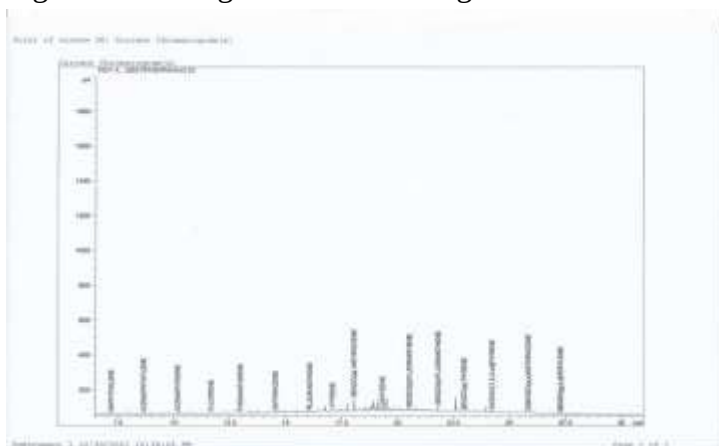


Fig 7: Chromatogram for boiled groundnut shell

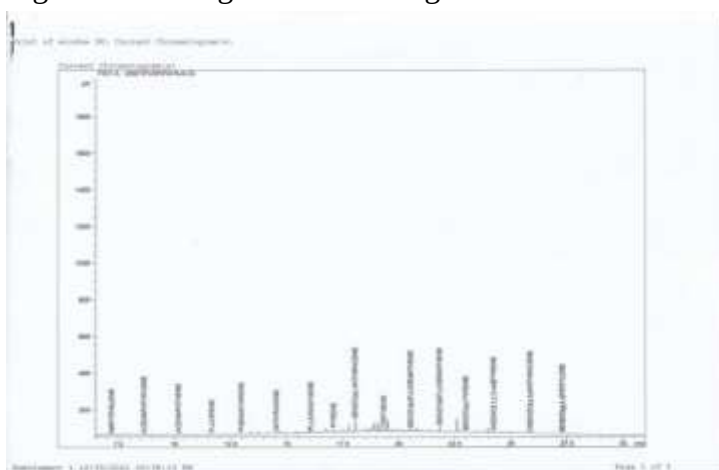


Fig 8: Chromatogram for boiled groundnut seeds

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