

Levels of Polycyclic Aromatic Hydrocarbons in Edible Vegetable Oil Sold In Umuahia, Main Market, Nigeria

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Abstract: *The aim of this work is to determine the levels of Polycyclic Aromatic hydrocarbons in both refined and unrefined oil sold in Umuahia, Nigeria. A total of 13 PAH standard (naphthalene, acenaphthylene, acenaphthene fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene) out of the US EPA priority PAH standards were used for PAH identification and quantification using Gas Chromatography with Flame Ionization Detector (GC-FID) fitted to a capillary column Restex 30 meter at 0.53mmID. The refined and unrefined oil were heated for 1 min and 5 mins to determine the effect of short time heating on PAH formation in the oil. Benzo[a]anthracene, (619.2µg/kg) was found in the refined oil. However, anthracene, (4824 µg/kg) and fluorene (1584µg/kg) were found in the unrefined oil heated for 1 minute. Most of PAH were below the instrument detection limit (1.44µg/kg).*

Keywords: *Polycyclic Hydrocarbon, Gas chromatography, Edible Vegetable oil, Carcinogenic, Silica gel.*

I. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of fused benzene-ring compounds formed during various domestic and industrial combustion as well as natural phenomenon such as volcanic eruption [1]. Polycyclic aromatic hydrocarbons also called poly-aromatic hydrocarbons or polynuclear aromatic hydrocarbons; are potent atmosphere pollutants that consist of fused aromatic rings and do not contain heteroatoms or carry substituents [2]. They have been classified as hazardous compounds of environmental concern due to their carcinogenicity and mutagenity [3], [4], [5] [6].

Consequently, sixteen PAHs compounds including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene etc have been listed as priority pollutants [7]. PAHs presence and concentration in food with respect to their effects in humans have attracted attention in recent times. Food processing methods like drying, smoking and cooking methods like grilling, roasting and frying are major sources through which PAHs contaminate processed foods [8] [9]. Direct contact of food materials with fire during processes like smoking, roasting and grilling have been established as sources contamination of food materials with high concentrations of PAHs [10].

PAHs are lipophilic, meaning that they mix more easily with oil than water. The larger compounds are less water- soluble and less volatile (that is, less prone to evaporate). Because of these properties, PAHs in the environment are found primarily in soil, sediments, and oily substances as opposed to in water or air [11]. Numerous PAHs are carcinogenic, making their presence in food and the environment a health concern. PAHs also occur in charbroiled and dried foods, and may form in edible oils by pyrolytic processes, such as incomplete combustion of organic substances. PAHs in foods can also result from petrogenic contamination. PAHs can arise naturally and as a result of anthropogenic activity. Exposure to PAHs occurs mainly by inhalation of air and by ingestion of food and drinking water [12] [13]. PAHs in food are mainly formed during industrial processing and food preparation, for example smoking, roasting, baking, drying, frying, or grilling [9].

Vegetable oils and fats are a significant source of PAH in the diet, either directly, as in the case of vegetable oils used for seasoning and margarine used for cooking, or indirectly by their incorporation into other foods such as the cereal-based products, biscuits and cakes [14]. The main route of PAHs uptake from food are oil and fat (50%), because of the strongly lipophilic nature of PAHs.

The levels of PAH in crude edible oils vary widely and refining (based on the deodorization step) reduces the concentration of the number of the lower molecular weight compound such as fluoranthene while no corresponding effect is observed for higher molecular weight PAH. This refining method has been reported to be widely used [14]. Concentrations of PAH in refined oils are in the range of µg/kg whereas in crude oils levels may exceed 2000µg/kg [15]. Since the existence and utilization of edible oil, there have been lack of inadequate literature or records on the PAH content of edible oil in Nigeria.

II. Materials And Methods

2.1 Sample Collection

Two different brands of vegetable oil used for the experiment was purchased from Umuahia main market. The first brand of vegetable oil was a local unrefined vegetable oil. Umuahia main market in Abia state Nigeria was grouped into fifteen clusters and 250ml of unrefined vegetable oil were collected from each cluster and homogenized to obtain a representative sample. The second brand of vegetable oil was a refined vegetable oil with 13/03/2012 as the production date and 28/03/2014 as the expiring date.

2.2 Sample Preparation

The samples were heated at 200°C for 1min and 5mins to determine the effect of heating period the formation of PAH. The unheated samples from each of the brand of vegetable oil were used as the control.

2.3 Extraction of PAHs from the Vegetable Oil Samples

2.3.1 Extraction with Dichloromethane (DCM)

The extraction of PAH was carried using dichloromethane (DCM). Exactly 5g of the oil sample was weighed in a beaker and 20ml DCM was added and stirred. The mixture was filtered and concentrated to 5ml. The extract was then turned into a 5ml GC-vial bottle for GC Analysis.

2.3.2 Extraction with Dichloromethane (DCM) and clean-up with Chromatographic Silica gel (100-200 Mesh) Size.

Exactly 5g of the oil sample was weighed into a beaker and 20ml of DCM was added and stirred. A 3g of chromatographic silica gel (100-200 mesh) was weighed and added to absorb non-hydrocarbon components in the oil. Another 3g of chromatographic silica gel (100-200 mesh) was placed on a cone filter paper in a funnel. The mixture was concentrated to 5ml and then turned into a 5ml GC-vial bottle for GC Analysis.

Gas Chromatographic Analysis

The detection of PAHs in the vegetable oil samples was conducted on a Gas chromatography with flame ionization detector (GC-FID) fused to a capillary column Restex 30 meter at 0.53mmID. The following conditions were maintained during the GC-Analysis: Detector temperature 330°C, Injector temperature, 250°C, Initial oven temperature, 100°C, Holding temperature/time, 4°C/min, Ramp at 320°C, Standard used, 610 PAH calibration mix A (methylene chloride), Volume injected, 0.8µL.

III. Results And Discussion

The chromatogram of 13 standard PAHs used to run the analysis which include naphthalene, acenaphthylene, acenaphthene fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene is shown in figure 3.1. These 13 PAH are among the 16 US EPA priority list. The US EPA proposed for the regulation, a set of the most important PAHs, commonly known as the 16 US EPA PAHs. Out of these 16PAHs, 13PAHs were used as a standard to run the analysis. Also, out of these PAHs, benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene are considered to be carcinogenic to humans [16] and [17]. Some PAHs are expected to be human carcinogens [18]. Several organizations have proposed maximum values for PAH in food products. Out of hundreds of PAHs, the most studied is benzo[a]pyrene, which is often used as a marker for PAH in ambient air and food [19] [7]. Apart from the use of benzo[a]pyrene as a marker, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene have high carcinogenic potential [17]. Naphthalene recently is considered a possible carcinogen to human by IARC (Group 2A) [20].

TABLE 3.1 shows the unrefined oil heated for 1 minute before the clean-up with chromatographic silica gel was found to have 158.4µg/kg fluorene and 4824µg/kg anthracene. However, appendix shows the chromatogram of the control sample of the unrefined oil, the unrefined oil heated for 1 minute and 5 minutes respectively. These chromatogram shows unidentified peaks in sample after clean up with chromatographic silica gel. The peaks may be other PAH whose standards were not among the ones used in this study. Nevertheless, other PAH in the same samples were below instrument detection limit (1.44 µg/kg). This may be due to the use of clean-up with chromatographic silica gel. The use of supercritical fluids as clean-up reagents during the extraction of PAHs from substances usually leads to low recovery of heavy PAHs [21]. This explains why the unrefined oil heated for 1 minute contained 158.4µg/kg fluorene and 4824µg/kg anthracene before the clean-up with chromatographic silica gel while the unrefined oil heated for 1 minute contained <1.44µg/kg levels of PAHs after the clean-up with chromatographic silica gel, as shown in Table 3.1.

The unrefined oil heated for 5 minutes, after the clean-up with chromatographic silica gel was found to

contain 619.2µg/kg benzo[a]anthracene, and two other unidentified peaks Benzo[a]anthracene have been shown to have a high carcinogenic potential [16] [17].

Since the local unrefined vegetable oil samples have not undergone refining processes such as deodorization and treatment with activated charcoal which can reduce the levels of PAHs significantly, some of the local vegetable oil samples were found to contain some levels of PAHs. This is because levels of PAHs in crude edible oils vary widely [22].

The concentration of PAH in the refined vegetable oil samples were bellow <1.44µg/kg levels of PAHs before and after the clean-up with chromatographic silica gel. This is because the occurrence of PAHs in vegetable oils is mostly related to their thermal treatment, in which combustion gases may make contact to the oil [23]. The main contributors of PAH uptake from food are oil and fat (50%), because of the strongly lipophilic nature of PAH [17]. The concentrations of PAHs in refined oils are in the range of few µg/kg whereas in crude oils, levels may exceed 2000µg/kg [15]. This is the case of the refined vegetable oil samples used in this study which were not heated to the combustion temperature. Also, the levels of PAHs in refined oil (based on the deodorization step and the treatment with activated charcoal), reduces the concentration of a number of the lower molecular weight compounds such as fluoranthene [24] [22].

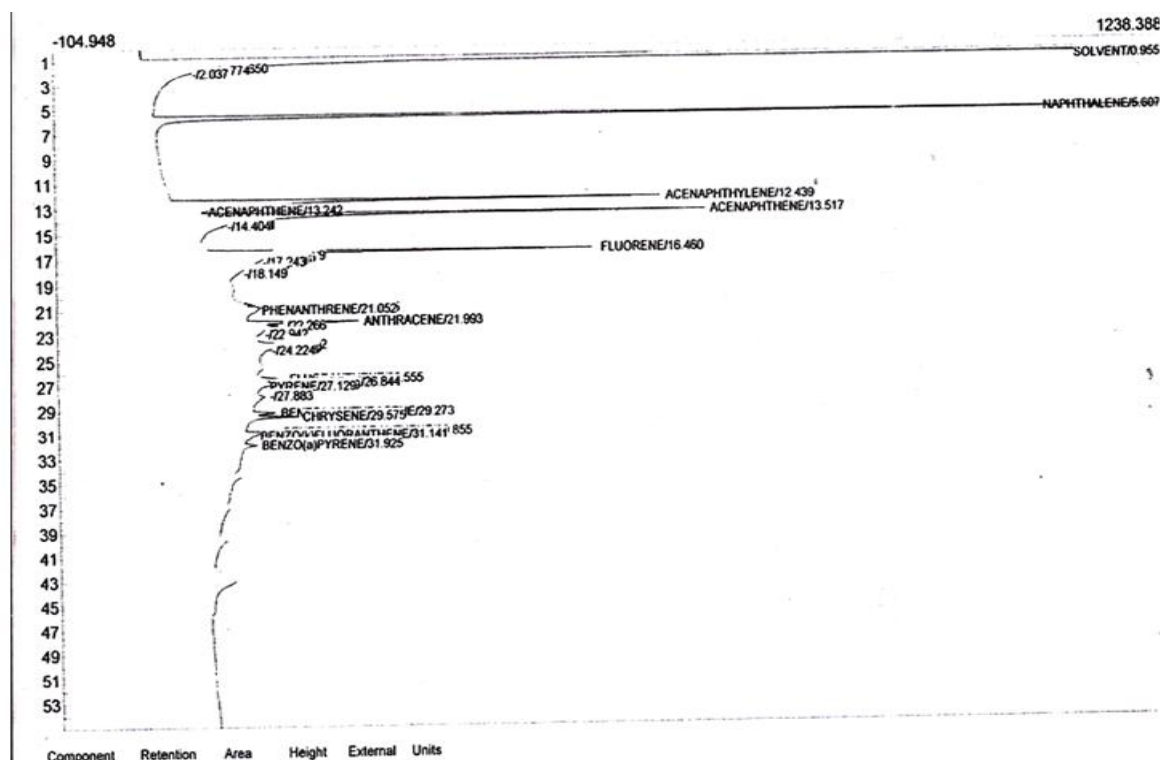


Fig.1: Chromatogram of the 13 Standard PAHs

TABLE 3.1: Values of the 13 PAHs Detected in the Vegetable Oil Samples

| Components | Local 0min Sample Conc. (µg/kg) | | Local 1min Sample Conc. (µg/kg) | | Local 5min Sample Conc. (µg/kg) | | Refined 0min Sample Conc. (µg/kg) | | Refined 1min Sample Conc. (µg/kg) | | Refined 5min Sample Conc. (µg/kg) | |
|-------------------------|---------------------------------|-------|---------------------------------|-------|---------------------------------|-------|-----------------------------------|-------|-----------------------------------|-------|-----------------------------------|-------|
| | Before | After | Before | After | Before | After | Before | After | Before | After | Before | After |
| 1 Naphthalene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 2 Acenaphthylene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 3 Acenaphthene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 4 Fluorine | <1.44 | <1.44 | 158.4 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 5 Phenanthrene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 6 Anthracene | <1.44 | <1.44 | 4824 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 7 Flouranthene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 8 Pyrene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 9 Benzo(a)anthracene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | 619.2 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 10 Chrysene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 11 Benzo(b)fluoranthene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 12 Benzo(k)fluoranthene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |
| 13 Benzo(a)pyrene | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 | <1.44 |

Note: Before means before adding chromatographic silica gel.

After means after adding chromatographic silica gel.
Unrefined 0min means the control sample of the unrefined vegetable oil.
Unrefined 1min means the unrefined vegetable oil heated for 1 minute.
Unrefined 5min means the unrefined vegetable oil heated for 5 minutes.
Refined 0min means the control sample of the refined vegetable oil.
Refined 1min means the refined vegetable oil heated for 1 minute.
Refined 5min means the refined vegetable oil heated for 5 minutes

Generally, the levels of PAHs detected in the vegetable oil samples were relatively low. Firstly, this is due to the fact that the vegetable oil samples were not heated for a long time at high temperatures. In comparison with the various samples of cooking oil analyzed in China [25], all the samples contained benzo[a]pyrene and dibenzo[a,h]anthracene. While refined vegetable oil samples which were also analyzed and measured contained 305µg/kg benzo[a]pyrene and 4565µg/kg dibenzo[a,h]anthracene. These various samples of cooking oil were obtained from various Chinese restaurants where the cooking oils were used for deep-frying at high temperatures [25]. Deep-fat frying produces desirable or undesirable compounds, changes the flavor stability, quality of the oil hydrolysis, oxidation and polymerization [26]. PAHs are part of the undesirable compounds produced. Processing of food (such as drying) and cooking of foods at high temperatures (grilling, frying) are major sources that generate PAHs [8] [9].

Secondly the vegetable oil samples obtained from the Umuahia main market had lower levels of PAHs because Umuahia city is not an industrial city or a highly environmental polluted city as compared to other cities in the world including China. This is because the concentrations of PAHs in all compartment of the environment vary, and it is regulated in most countries in the world [27]. PAHs are ubiquitous environmental contaminants formed and released during incomplete combustion or by industrial processes. PAHs can arise both naturally and as a result of anthropogenic activities (i.e. human activities). These anthropogenic activities are much more important contributors of environmentally hazardous compounds [17]. PAHs are also volatile compounds. The occurrence of PAHs in food is due to environmental contamination (deposition of airborne particulates on crops or growth in contaminated soil), technological processing or home cooking and in low amounts from contaminated packaging materials [28] [6] [29] [30]. Examples of anthropogenic activities in the environment which influences the formation and the release of PAHs in most countries and cities unlike the Umuahia city include: stubble burning [31], spreading of contaminated sewage sludge on agricultural fields [32], exhausts from mobile sources (motor vehicles and aircrafts), industrial plants (e.g. aluminum foundries, incinerators), domestic heating with open fire places, burning of coal for thermal and electric energy and oil pollution of surface waters and soils etc. [22].

IV. Conclusion

The concentrations of PAHs in the oil samples were relatively low and most of the concentrations were below the instrument detection limit. It could therefore be concluded that during the processing of foods with the use of vegetable oil, vegetable oil should not be heated for a long period of time at high temperatures to prevent the occurrence and the formation of PAHs.

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Appendix

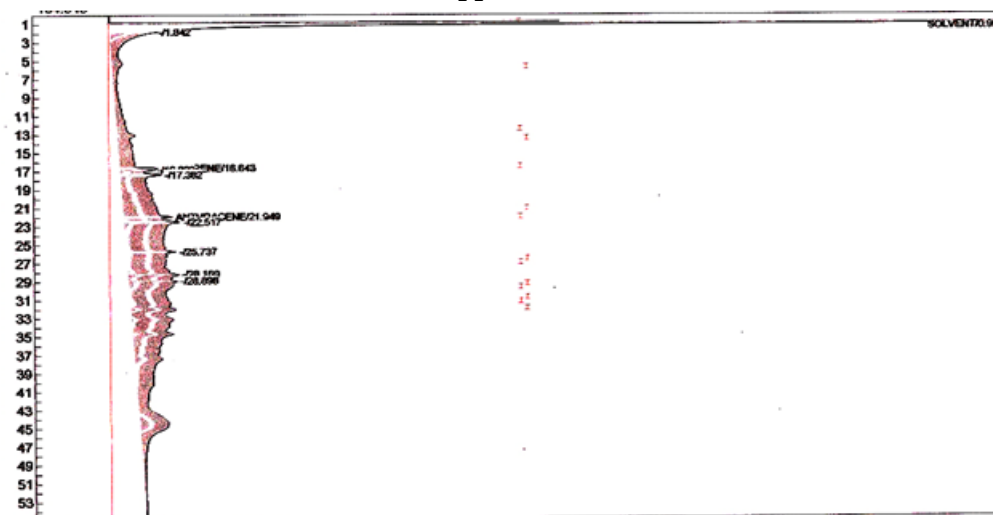


Fig.3: Chromatogram of the control sample of the unrefined oil after the clean-up with chromatographic silica gel

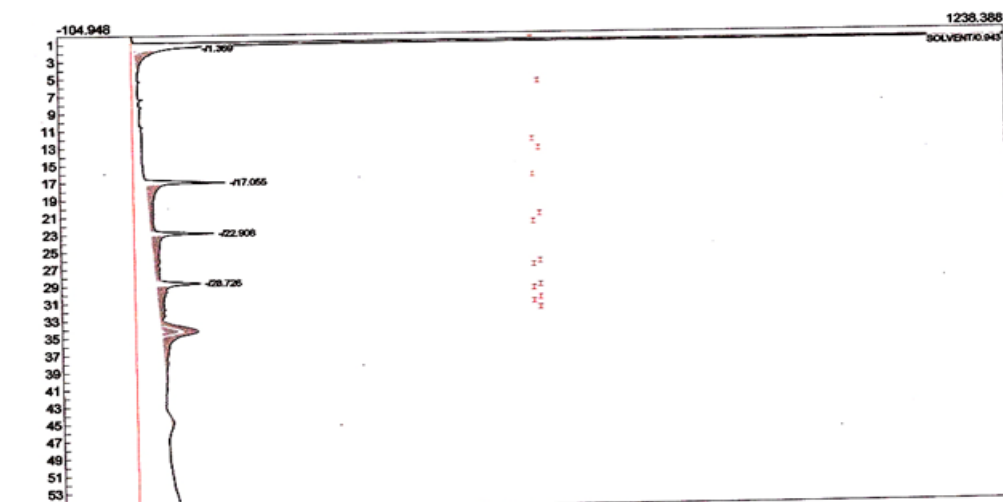


Fig. 3: Chromatogram of the control sample of the unrefined oil after the clean-up with chromatographic silica gel

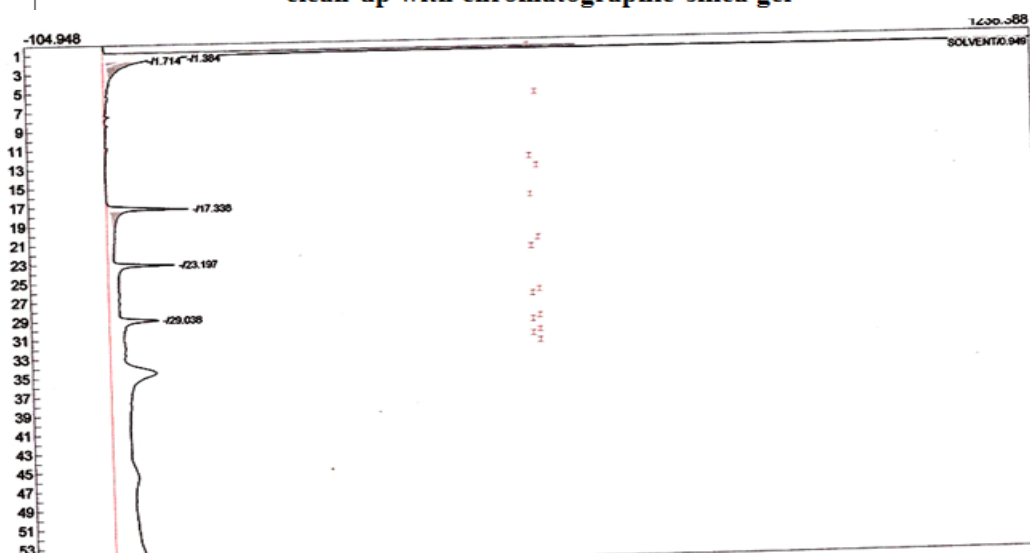


Fig.5: Chromatogram of the unrefined oil heated for 5minute after the clean-up with chromatographic silica gel

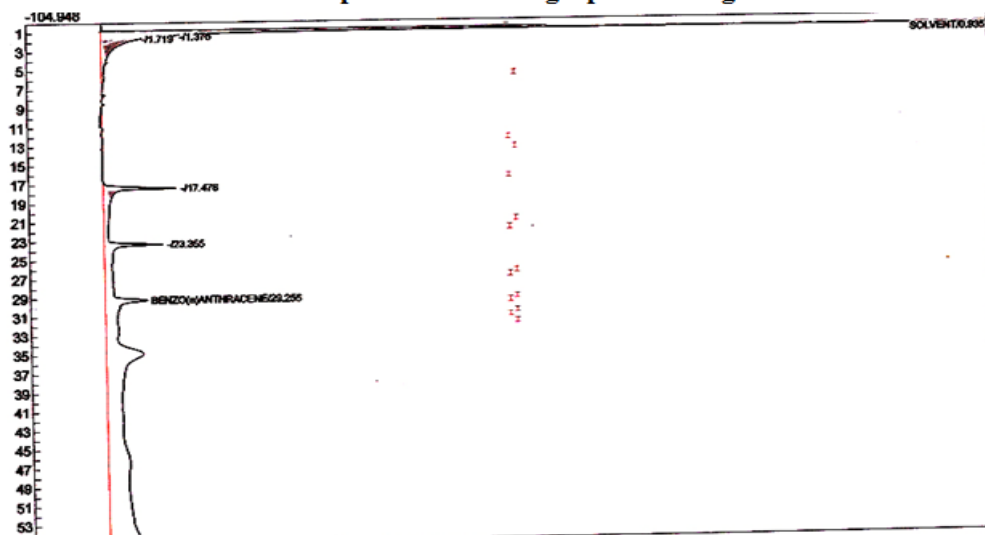


Fig.5: Chromatogram of the unrefined oil heated for 5minutes after the clean-up with chromatographic silica gel