# Essential Amino Acid Profile and Polycyclic Aromatic Hydrocarbons (Pahs) Of *Clarias Gariepinus* Smoked with Different Firewood

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## ABSTRACT

Safety of food is of great concern worldwide and y is critical to consumers due to its public health implication. This study examined the essential amino acids profile and concentration of polycyclic aromatic hydrocarbon of Clarias gariepinus smoked with different firewood with a view of determining its quality and safety. This study was carried out in two phases; the first was pilot survey using structured questionnaire to identify the types of firewood used by fish processors at Hadejia, Jigawa State, Geriyo Adamawa State and Laranto Plateau State. While the second phase was a laboratory work to determine the essential amino acid profile and concentration of polycyclic aromatic hydrocarbons of the sampled fish species. Smoked and fresh *Clarias gariepinus* samples were collected from the study areas. Three types of woods reported through the questionnaire as commonly used by fish processors were also used to smoke the fresh *Clarias gariepinus* purchased. Amino acid analysis was performed on reverse phase-high pressure liquid chromatography (HPLC) (Buck scientific BLC 10/11 Las Vegas USA) equipped with UV 338nm detector while PAHs analysis was conducted using High performance liquid chromotography (HPLC) Hewlett Packed 589 0 series II, coupled with flame ionization detector (FID) (Hewlett Packard, Wilmington, DE, USA). The questionnaire results showed that majority of the respondents (64.58% ) in Hadejia used wood from Neem tree, in Geriyo majority (63.26%) used wood from Chew tree and in Laranto majority (58.00% ) of the respondents used wood from Tallow tree for smoking their fish. The results of essential amino acids analysis showed that all the fish samples have essential amino acids. Leucine, Phenylalamine, Valine and Arginin are the most concentrated essential amino acids in the samples while Threonine and Methionine are the least detected. The results from analysis of Clarias gariepinus for polycyclic aromatic hydrocarbons showed that thirteen PAHs were detected in the smoked fish samples although, Benzo(a)pyrene, Benzo(a)athracene and Benzo(b)flouranthene were not detected in the fresh fish sample and they were the least detected in smoked fish samples, this implies that they are introduced during smoking process. Acenephene, Acenephylene, Flouranthene and Pyrene are the most concentrated PAHs in the smoked fish samples. All the PAHs detected in this study are within the normal residue limit specified by the world health organization.

**Key words:** Amino acid, polycyclic aromatic hydrocarbon, *Clarias gariepinus*

## INTRODUCTION

Fish is a major source of food for human populations, providing a significant portion of the protein intake, in the diets of a large of people, especially in developing countries (Da Silva, 2002). Fish proteins comprise all the ten essential amino acids which are: lysine, leucine, arginine, methionine, alanine, histidine, phenylalanine, isoleucine, threonine and tryptophan in desirable quantities for human consumption. This accounts for the high biological value of fish flesh, which also provides minerals, iodine, vitamins and fat. Fish cooks easily, offers palatable taste and flavour and is easily digestible. Fish is consumed either as a freshly caught fish or from those that have been preserved in some form (Da Silva, 2002). However, fish is an extremely perishable commodity, spoiling soon after death, due to enzymatic and microbial activities. Some factors responsible for this include the prevailing high temperatures in Nigeria and the facilities for processing; Storing and distributing the fish caught are frequently inadequate. Food smoking belongs to one of the oldest technologies of food preservation which mankind has used in fish processing. Smoking has become a means of offering diversified, high value added products as an additional marketing option for certain fish species where fresh consumption becomes limited (Gómez *et al.,* 2009). Traditional smoking techniques involve treating of pre-salted, whole or filleted fish with wood smoke in which smoke from incomplete wood burning comes into direct contact with the product, this can lead to its contamination with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed (Estaca *et al*., 2011). The actual levels of PAHs in smoked foods depend on several variables in the smoking process, including type of smoke generator, combustion temperature, and degree of smoking (Garcia and Simal, 2005). The composition of the smoke and the conditions of processing affect the sensory quality, shelf life, and wholesomeness of the product. Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke; mainly PAHs, derivatives of PAHs, such as nitro-PAH or oxygenated PAH and to a lesser extent heterocyclic amines (Stołyhwo and Sikorski, 2005).

Fish smoking has gained a lot of ground in the field of fish processing and it is commonly practiced by small, medium and large scale processing firms. The smoke which is produced either from wood or charcoal fuel contains polycyclic aromatic hydrocarbons (PAHs) such as Benzo(a)pyrene, Benzo(a)athracene and Benzo(b)fluoranthene and more, which are a large group of organic compounds with sufficient toxicological evidence for mutagenic and carcinogenic effect (Ramalhosa *et al*., 2009). Most individuals are predominantly exposed to PAHs from dietary sources such as smoked fish, grilled beef, bush meat etc (Bordajandi *et al.*, 2008). PAHs also are bio-available to marine species through the food chain, as water borne compounds and contaminated sediments. Since they are lipophilic compounds, they can easily cross lipid membranes and have the potential to bio-accumulate in aquatic organisms. Despite fish and sea food representing only a small part of the total diet in most human, their contributions to the daily intake of PAHs in some individuals may be comparatively important (Domingo *et al.,* 2007).

The contamination of foodstuffs by PAHs can occur at source through atmospheric deposition on harvested fish (Culotta *et al.,* 2002), or from preservation of food by smoking, drying and cooking procedures. This contamination is enhanced during smoking and intense thermal processing (Chen and Chen, 2001). During intense thermal processing, the contamination occurs by direct pyrolysis of food nutrients (Orecchio and Papuzza, 2008). The PAHs are also deposited from smoke produced through the incomplete combustion of different thermal agents.

Safety of food is of great concern globally. PAHs residues if present in food above the maximum residue limit (MRL) pose a serious threat to public health. Consumption of these PAHs at certain levels becomes detrimental to human health (Chen and Chen, 2001).

## MATERIALS AND METHODS

This research was conducted in two phases, the first phase is a pilot survey to ascertain the types firewood used by fish processors in Hadejia, Jigawa State, Geriyo Adamawa State and Laranto in Plateau State using structured questionnaire and the second phase was Laboratory work to determine the essential amino acid and polycyclic aromatic hydrocarbon compositions of the sampled fish species (*Clarias gariepinus*).

### Collection of Fish Samples and Wood for Fish Smoking

Smoked *Clarias gariepinus,* was purchased from fish processors (smokers) at Hadejia, Geriyo and Laranto fish markets in Jigawa, Adamawa and Plateau State, respectively. The wood type used at the three locations were collected and brought to the Department of Forestry and wildlife, Modibbo Adama University, Yola for proper identification. The wood types were purchased and used for smoking fresh samples of the fish species at Binyaminu Usman Polytechnic, Hadejia. Fresh samples of *Clarias gariepinus* were purchased at the landing site of Hadejia River and transported in fish box to Hadejia where smoking of the fish was done.

### Smoking Technique

The fresh *Clarias gariepinus* was degutted, washed and allowed to drain. Initial weight of the samples was

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measured prior to smoking; the samples were placed on smoking kiln. The samples were subjected to laboratory analysis to determine the essential amino acid and polycyclic aromatic hydrocarbon compositions of the fresh and smoked fish samples.

**Determination of amino acids**.

The sample (30mg) was hydrolyzed with 6N HCI at 110°C for 24h. Amino acid analysis was performed on reverse phase-high pressure liquid chromatography (HPLC) (Buck scientific BLC 10/11 Las Vegas USA) equipped with UV 338nm detector. A C18, 2.5 x 200mm, 5um column and a mobile phase of 1:2:2 (100mM sodium phosphate, pH 7.2: Acetonitrile: methanol) was used at a flow rate of 0.45 mL/minute and an operating temperature of 40$^{o}$C. Mixed standards were analyzed in a similar manner for identification. All chemicals and standards were of analytical grade from Sigma-Aldrich, St. Louis, MO. Peak identification was conducted by comparing the retention times of authentic standards and those obtained from the samples, these data were integrated using peak Simple chromatography data system processor; (Buck SCi. chromatopac data processor).

**Determination of PAHs in fresh and smoked Fish Tissue**

Two gram (2g) of each of the homogenized fish samples were thoroughly mixed with anhydrous $Na_2SO4$ salt to absorb moisture and then extracted with unspecified quantity of analytical grade dichloromethane ($CH_2CL_2$). The dichloromethane extract was cleaned up by passing through a column packed with anhydrous $Na_2SO4$ salt. The resulting extract was concentrated on a rotary evaporator to give an oily residue; which was again dissolved in 1ml $CH_2CL_2$ and 1μL was injected into the HPLC for analysis. The HPLC used was Hewlett Packed 589 0 series II, coupled with flame ionization detector (FID) (Hewlett Packard, Wilmington, DE, USA). The identification of PAHs was based on comparison of the retention times of the peaks with those obtained from standard mixture of PAHs (standards supplied by instrument manufacturer). Quantification was based on external calibrations curves prepared from the standard solution of each of the PAHs.

**Data Analysis**

The data collected were subjected to graphical representation (Barchart) and One-way analysis of variance (ANOVA). The Least Significance Difference (LSD) was used to test for the difference between the treatments values with significance difference at $p \leq 0.05$. Microsoft Excel Data Analysis and VPAST were used for analyses the data.

## RESULTS AND DISCUSSIONS

Amino acid composition of smoked *Clarias gariepinus* is presented in Figure 1. It was observed that among the essential amino acids, Leucine, Valine and Arginine were the most abundant in all smoked *Clarias gariepinus* samples. The highest value of Leucine, Valine and Arginine recorded are: 9.41 for Leucine, 8.81 for Valine and 8.12 for Arginine. Among the non-essential amino acids, Glutamic acid and aspartic acid were the most abundant amino acids in all the smoked *Clarias gariepinus* samples and make up to values 9.45 and 8.65 respectively. Cystine and Proline has the lowest value of non-essential amino acids of 1.96 and 3.27 in all smoked *Clarias gariepinus* samples.

All essential amino acids that are very important for human body are all present in the fish. These essential amino acids are lysine, leucine, valine, isoleucine, threonine, phenyl alanine, methionine, histidine and tryptophan. Amino acids are also important in healing processes and the composition of amino acids in fish is required by man for good health. Therefore, people can acquire essential amino acids in abundance and proper balance by eating fish (Osibona *et al.,* 2009). The essential amino acids cannot be manufactured in the human body, but can be obtained from food. Deficiency in the essential amino acids may hinder the healing recovery process (Osibona *et al.,* 2009).

Leucine promotes the healing of bones, skin and muscle tissues. Isoleucine is necessary for haemoglobin formation, stabilizing and regulating blood sugar and energy. Glycine which is one of the major components of human skin, collagen, together with other essential amino acids such as alanine form a polypeptide that will promote re-growth and tissue healing (Mohanty *et al*., 2014).

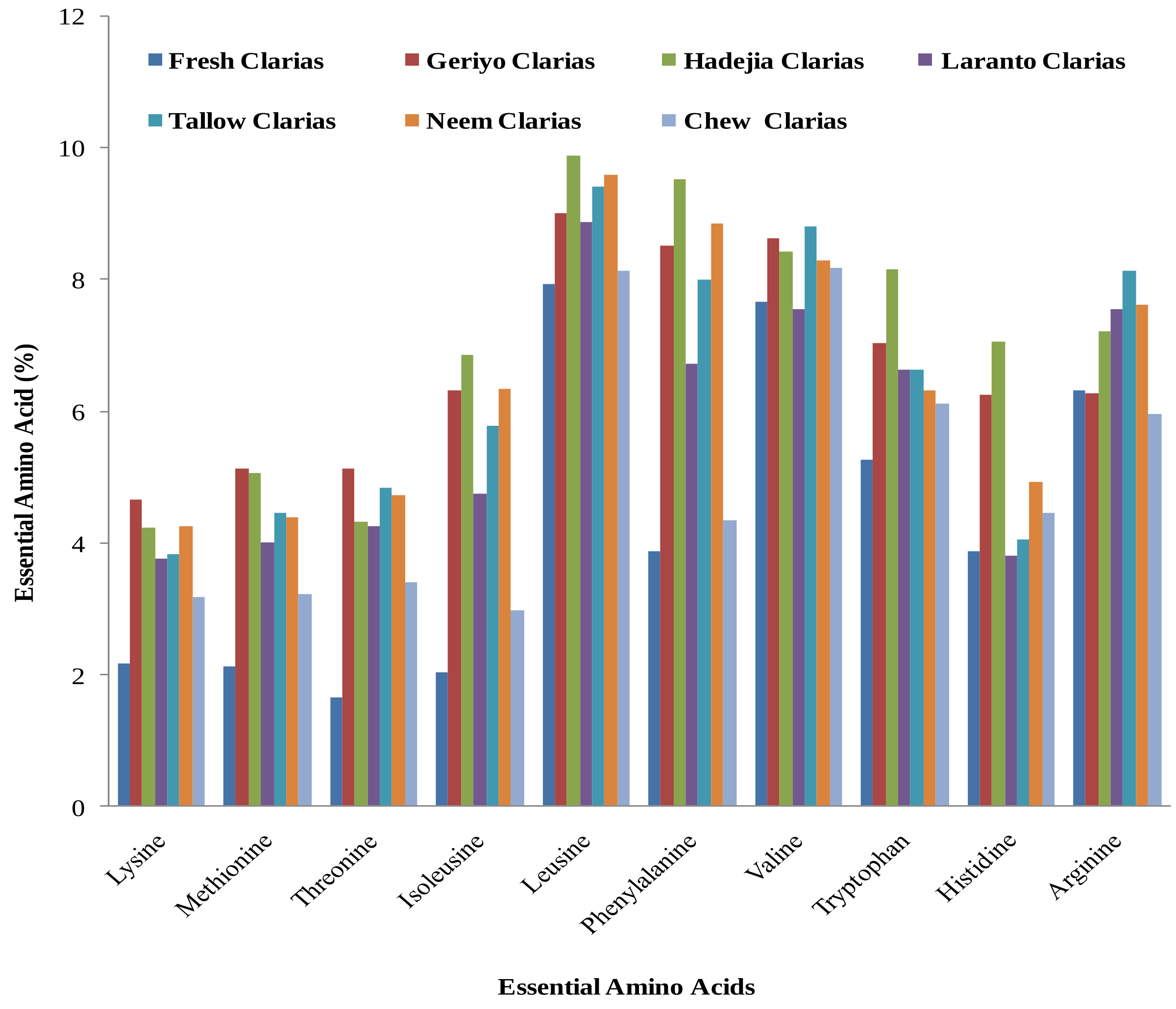


**Figure 3. Essential Amino Acids of Fresh and Smoked *Clarias gariepinus***

The results obtained from laboratory analysis of *Clarias gariepinus* for Polycyclic aromatic hydrocarbon profile showed that thirteen of the hydrocarbons although in the fresh fish samples Benzo(a)pyrene, Benzo(a)athracene and Benzo(b)flouranthene were not detected and these hyrocarbons were having lowest values in the smoked and purchased *C. gariepinus*.Highest Acenaphylene, pyreneand chrysene were recorded in *C. gariepinus* purchased at Laranto while the lowest were from the fresh (Figure 2) and these have the highest concentrations compared to other hydrocarbons.

Due to their carcinogenic activity, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Human exposure to PAHs occurs in three ways, inhalation, dermal contact and consumption of contaminated foods. Diet is the major source of human exposure to PAHs as it accounts for 88 to 98% of such contamination (Farhadian *et al.*, 2011). Processing of food at high temperatures (grilling,roasting,fryingandsmoking) are major sources generating PAHs. Levels as high as 200µg/kg has been found for individual PAH in smoked fish and meat samples.

The actual levels of PAHs in smoked foods depend on several variables in the smoking process, including type of smoke generator, combustion temperature, and degree of smoking (Garcia and Simal, 2005) agreed with the findings of this study that different wood sources have different PAHs. Omodara *et al*. (2019) had also reported that the total PAHs found in all smoked mud fish in this study were higher than what was found in fresh (control) fish which also agreed with the findings of this study. Amount of PAHs in samples smoked with wood was significantly different from samples smoked with charcoal. They also documented 24 PAHs which were hgher that 13 documeted in this study in Mudfish. In their work, Silva *et al*. (2011), in most of the smoked fishes studied, benzo (a) pyrene was not detected except for the oven dried *C. senegalensis*which was found to have a level of 5.6 µg/kg, which exceeds the 5.0 µg/kg maximum level for smoked meat and fish established by the European Commission (Regulation (EC) No 208/2005). The results reveal that the fish samples smoked by the different methods do not constitute a health risk, as the levels of the benzo (a) pyrene are below or lower than maximum levels regulated by the European Commission**.**

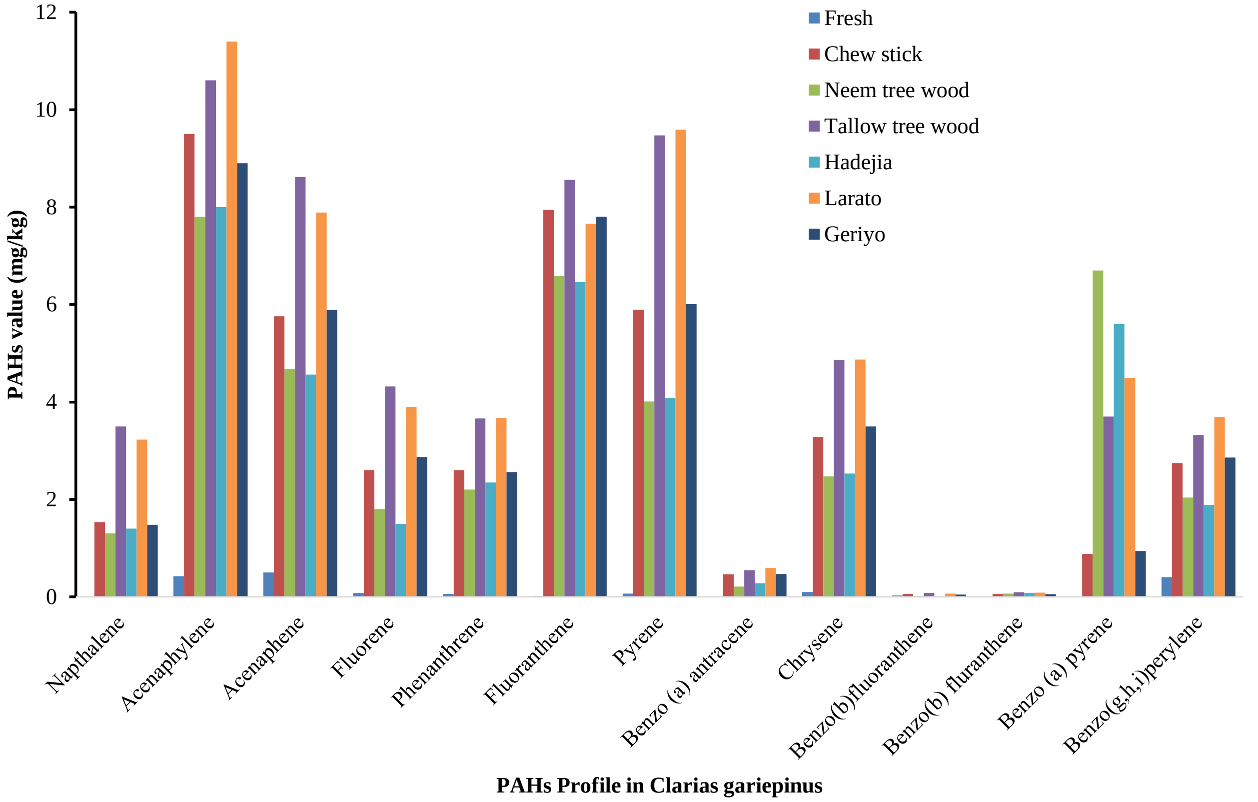


**Figure 15. PolyAromatic Hydrocarbon Profile from fresh and differently smoked *Clarias gariepinu*s**

Fish and marine invertebrates may naturally contain small amounts of different PAH absorbed from the environment (Stolyhwo and Sikorski, 2005). In this study Benzo(a)pyrene, Benzo(a)athracene and Benzo(b)fluoranthene are not detected in fresh fish samples, this indicated that the values of Benzo(a)pyrene, Benzo(a)athracene and Benzo(b)fluoranthene detected in the smoked fish samples were introduced during smoking. Acenaphylene, Acenaphene, pyrene, Fluoranthene and Benzo(a)pyrene are the most concentrated Polycyclic aromatic hydrocarbons in all smoked fish samples. The results of total polycyclic aromatic hydrocarbons showed that, there is significant different ($p<0.05$) between PAHs in all fresh and smoked fish samples. In smoked fish samples, there is significant different between Clarias smoked with Chew, Clarias smoked Neem and Clarias smoked with Tallow wood.

The accumulation and depuration of PAHs in fish tissues is a function of the fat content, route and duration of exposure, environmental factors, difference in species, age, sex, and exposure to other xenobiotics (Deb, 2000, Dhananjayan and Muralidharan 2012).

The effect of smoking is clearly seen from Table 22 . For example, whereas some of the PAHs are non-detectable in the fresh samples, they were found in the smoked samples of the fish species. Also, the total amount of the PAHs levels in the smoked fish samples increased more than that for the fresh samples. This means that smoking the fish samples definitely was responsible for the introduction of the various PAHs in the fish samples. Therefore, PAHs, which ordinarily would not have been contacted by eating the fresh fish, now contains PAHs as a result of smoking. This implies that the people, who are fond of eating smoked fish, are at risk of the problems associated with PAHs (Jude *et al*.,2012).

Chukujindu *et al*.,(2015) reported that fresh water fish species tend to have higher concentration of PAHs than the marine species due to the fact that many human activities are centered on freshwater ecosystems rather than marine environments as a result of their closeness to lands.

## CONCLUSION

Amino acids are important in healing and metabolic processes in humans. The essential amino acids needed by man are present in fish. Therefore, people can get enough essential amino acids by eating fish. The PAHs values obtained in this study were lower than the limit set by the Joint

FAO/ WHO which made the fish samples safe for consumption and may not pose the problem of carcinogenicity usually associated with smoked fish.

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