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PROXIMATE ANALYSIS AND DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN PROCESSED ANIMAL SKIN SOLD IN MAJOR MARKETS IN SOUTHWEST NIGERIA.

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ABSTRACT

This study aimed at determining the nutritional and level of chemical contaminants (heavy metals and Polyaromatic Hydrocarbons PAHs) incommonly consumed animal skin sold in major markets in South-West, Nigeria. Proximate analysis (crude protein, moisture content, carbohydrate, ash content, oil content, nitrogen value and crude fibre) and heavy metals and PAHs concentrations were determined to evaluate the nutritional composition and safety of regular consumption of these products and to validate the notion that animal skin as a source of protein, can replace meat in meals. Samples of animal skin were purchased from three major markets in Lagos and Ogun State. Proximate composition was determine using standard analytical methods, while levels of heavy metal (Fe, Pb, Cd, Cr and As) and PAHs were using Atomic Absorption Spectroscopy and GC-MS respectively.The results of proximate composition were as follows (%); for the brown samples; the moisture content ($\leq$81.40±1.80, ash content ($\leq$0.94±0.01, oil content ($\leq$1.60±0.01, crude protein ($\leq$3.75±0.85, crude fibre ($\leq$1.25±0.01) and carbohydrate ($\leq$14.51±1.20). The concentrations of heavy metals detected in the samples were as follows (mg/kg); Fe $\leq$0.068±0.001, Cd $\leq$0.006±0.001, Pb$\leq$0.004 ±0.001 and Cr$\leq$0.004±0.001, all were below the maximum permissible limit set by WHO and USEPA while, Arsenic was below the detection limit of the instrument. Seventeen PAHs were detected in the samples and all were above the maximum permissible limits with high health risk. It is therefore advised that cow skin be discouraged and more awareness created so that people will know the dangers to health posed by this harmful practice.

Keywords: Animal skin, Polyaromatic Hydrocarbons, Heavy metals, proximate analysis, GC-MS

INTRODUCTION

Food safety and sustainability of life can be threatened by contaminants in foods and one of the major channels through which food is contaminated is food processing. Food processing can have negative impact on the food product, and this is a worldwide challenge [1].Foods are processed in order to improve the taste, preservation, readily edible and remove unwanted substances. One of the foods products that are vulnerable to contamination during processing in Africa is animal skin. Shortage and high cost of meat has made people, especially in developing countries to find alternative in animal skin as replacement for meat [2]. In Nigeria, many believe that a meal is not complete without animal skin as it is included in major delicacy and in preparation of several stews especially at several public outings [3].

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The processing of animal skin, also called singeing involves tenderizing the skin in hot water followed by shaving with razor blade or burning of the hair on the skin using firewood, tyres, plastics and spent engine oil mixed with firewood [2]. The burnt animal skin are scraped to remove ash and thereafter boiled in water for about one hour to obtain the finished product [4]. Contaminant may be incorporated into the animal skin during singing process, from the shavers used in removing the cow skin hair [5] and materials used in singeing [2, 3, 6, 7]. The residue of the chemical substances administer to diseased animal to treat ailment are retained in the hide even after slaughtering and processing [8].

Some of the toxic chemical contaminants that have been reported in processed animal skin are polyaromatic hydrocarbons (PAHs), dioxins, furan, and benzene [2, 6] and toxic metals especially lead [7]. Food processing of fats and proteins that involves smoking or high-temperature cooking is one of the major sources of PAHs contamination in foods. Burning of polystyrene plastics may release styrene vapour, which can readily be absorbed by the cow skin. Long-termexposure to styrene affects the central nervous system, causes headaches, depression, fatigue while short-term exposure can cause eye and throat irritation. Polychlorinated biphenyls (PCBs) and heterocyclic amines are also released when plastics are burnt, contaminating the cowhides [9, 10, 11].

Potential known human health effects associated with singeing of cowhide include; short-term tissue irritation (such as skin, respiratory, eyes and gastrointestinal), decreased fertility, developmental neurological effects and renaltoxicity amongst butchers exposed to relatively high levels of PAHs [11]. The processorsare also at risk because of the associated health hazards through inhalation of these toxic compounds. Significant levels of PAHs have been reported in processed foods [12]. Many of these PAHs(benzo[a]pyrene, benzo[a] anthracene, dibenzo[a,h]anthracene and chrysene) have been reported to possess carcinogenic and genotoxic properties [13, 14]. PAHs may result in biochemical disruptions of DNA and cell damage in the body [15] and can lead to mutations and development of tumors and cancerous cells [16, 17].

Health risk assessment of contaminants and residues in foods need to be monitored regularly. This is germane in other to ensure the safety of consumersin agreement with regulatory limits. Additionally, they can also help to reveal and determine new or unexpected emerging chemical substances [18].

The aim of this study is to determine the proximate composition, level of some heavy metals and Polyaromatic hydrocarbons (PAHs) in animal skin sold in major markets in Southwest, Nigeria. This is to ascertain the safety and nutritional value of this fondly eaten material. The findings of this study provide

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information on the level of contaminants in cow skin and give answers to the pertinent question" How safe is the processed animalskin sold in the market?

MATERIALS AND METHODS

Sample Collection

Six samples of brown animal skinwere purchased from different points of sale in three selected major markets in Ogun and Lagos States. This includes Ita ale market (Site A), central market (Site B) and Main Market (Site C).The purposive sampling method was adopted because the samples were sold at their points of sale in the market rather than at clusters. About 0.6kg of the animal skin was purchased from each seller and the samples were wrapped in aluminum foils,labeled, and transported to the laboratory in an ice-chest. The samples were kept in the freezer at 4ºc before analysis.

Laboratory Analysis

Proximate Analysis

The analytical methods by Association of Official Analytical Chemists [19], for proximate analysis were adopted in this study. Moisture was determined using hot air oven at 105°C, until constant weight. Crude protein was determined usingKjeldahl method, total nitrogen was multiplied by a protein factor of 6.25. Dietary fiber was determined using the enzymatic gravimetric method. Ash was determined using gravimetric method by incinerating the samples at 550°C, until constant weight. Total carbohydrate was determined according to the difference method by calculation

Heavy Metals Analysis of Samples

The concentrations of selected heavy metals (Lead (Pb), Cadmium (Cd), Copper (Cu), Iron (Fe) and Arsenic (As) in the animal skin samples were determined using Buck Scientific Atomic Absorption Spectrophotometer (model 210A) after acid digestion. One (1g) of each sample was digested with 10 mL of Aqua regia (1:3 HNO_3 and HCl) [19].Standard solutions of the metals of interest were run in the instrument (AAS) for calibration before analysis.

Extraction of PAHs and Silica Gel Cleanup

The cow skin samples were allowed to dry and homogenized to powder, 5g of the homogenized sample was weighed and transferred into a beaker and 100ml of hexane: Acetone (1:1) was added. The sample was placed in an ultrasonic bath for 20 minutes and the mixture was allowed to settle and the solvent layer was decanted, the rotary evaporator was used to concentrate the mixture to 2ml and it was cleaned using a silica gel.

This method is based on EPA method 3630C.Silica gel was activated by heating at 130oc for 16 hrs. It was then stored in activated silica gel in a desiccators. A glass column was packed with 5g of silica gel and 1g of Anhydrous Na_2SO_4 was added. 20 ml of N-hexane was added to the column and eluted into

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beaker. The 2ml sample extract was added to the top of the column quantitatively. Another 10ml of n-hexane was added to the column and eluted to waste. Before the column head dried out, 10 ml (1+1) Dichloromethane+Hexane was added and the eluent was collected.The eluent was then concentrated to 2 ml using a rotary evaporator and PAHs qualification and quantification was assessed using gas chromatography with mass spectrophotometry (GC-MS) [20].

Determination of PAHs

The identification and quantification of the PAHs in the extracts were carried out using gas chromatography (GC-MS Spectrometer model 6800) according to the method described by TNRCC (1997) available at the Nigerian Institute of Oceanography and Marine Research, Victoria Island, Lagos, Nigeria. The identification was based on the comparison of the retention times of the peaks with those obtained from the mixture of PAHs standards while; quantification was based on external calibration curves prepared from the standard samples of each of the PAHs.

Statistical Analysis

Data generated from proximate, heavy metal and polycyclic aromatic hydrocarbon analysis were evaluated using coefficient of variation statistical method, and the means were declared significantly different at 5% ($P \leq 0.05$) from [21].

RESULTS AND DISCUSSION

The result of proximate analysis is presented on Table 1 while concentration of heavy metals (Iron, Lead, Cadmium, Chromium and Arsenic) in the samples is presented in Table 2. Proximate analysis and the concentration of heavy metals were determined in the collected animal skin samples. This is to ascertain the concentration of these contaminants and their safety for consumption

Proximate Analysis

The results of proximate analysis of the collected animal skin samples from the three sites follow similar trend which is; moisture content>carbohydrate>crude protein>oil content>crude fibre>ash content. Moisture is sometime used for the estimation of the quality of food andits one of the main factors for storage consideration due to the proliferation of microorganisms, such as fungi and mold. Total nitrogen consisted of protein nitrogen and little non-protein nitrogen. Ash content is the amount of total mineral residue left after incineration of samples until constant weight [22]. The study of moisture, protein, fat and ashcontents of animal skin have a great significance for consumers and scientists, apart from knowing thenutritive value, also aids in understanding its better processing and preservation [23].

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Table 1: Result of proximate analysis of the animal skin samples

Parameter	**A**	**B**	**C**
Moisture content @ 105^{O}C	81.4 ±1.80	81.11 ±1.00	79.92 ±2.10
Ash content @ 450^{O}C	0.94 ±0.01	0.82 ±0.01	0.81 ±0.01
Oil content	1.34 ±0.03	1.36 ±0.01	1.6 ±0.01
Crude Protein (%N*6.25)	3.75 ±0.85	3.75 ±0.02	1.68
Crude Fibre	1.23 ±0.01	1.25 ±0.01	1.18 ±0.01
Carbohydrate	10.74 ±0.05	11.11 ±0.03	14.51 ±1.20

The moisture and ash contents of all the collected samples from the three sites followsimilar trend; C<B<A. Organisms that encourage food spoilage flourishwell in foods with high moisture contents, thereby reducing the shelf life.Samples with high moisture content have a low shelf life because microorganisms can thrive and grow more in food with high moisture content[24, 25]. Ash content may reflect the amount of mineral or metallic composition in a sample [26].Based on the fact that the concentrations of metallic content in the analyzed samples are low (Table 2), it can be inferred that the result of ash content of the samples is a reflection of the mineral content. High ash content of hides can be attributed to the heat intensity generated from the processing methods. Diets with low fibre could cause constipation and eventually lead to disease of the colon like pile, appendicitis and cancer [27].

Oil content follow this trend; A<B<C, similar to carbohydrate content. Though, protein is detected in the animal skin samples, the amount of carbohydrate in the samples is higher than protein content. This is not in line with what has been reported by [28] but it's similar to the report of [29]. It is also observed that the animal skin samples have high carbohydrate content than protein, which contradicts what has been reported by [28, 29]. The protein content of the cow skin samples are low,this may be attributed to heat used in singeing the cow skin, resulting

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in degradation of the essential amino acids of protein [30].

Result of Heavy Metals

Heavy metals determined in this study were detected in all the animal skin samples except As. The concentration of all the heavy metals was below the permissible limit of heavy metals in food as set by USEPA and W.H.O. The concentrations of Fe and Cr in the animal skin samples from sites A and C are similar, while Cd concentrations in the three sites follow this trend; A<B<C.

The result of Pb in this study is lower than [31] report with a value of 1.99±0.86 ppm, but almost similar to the result of [29] with a value of 0.00-0.71 ppm while [5] had a very high value of 1.54 $mgkg^{-1}$. The results of Cd for this study is low compared to the result of [29] (0.000-0.240 ppm), but when compared to WHO permissible limit, the level of Cadmium in the samples were found to be at a close range as the value which is (0.003) ppm and the USEPA maximum permissible limit of (0.005) ppm,

According to Ayanda et al. [31], the detection of Cadmium in animal skin could be as a result of the animal skin coming into contact with both soils and particles dispersed by the wind. Cadmium exposure is toxic to the kidney; it causes bone demineralization [33]. It also causes cancer of the bladder, pancreas, gall bladder and testes [33]. According to Okiei et al. [5] report, ingestion of Chromium may lead to vomiting, diarrhea, blood loss in the gastrointestinal tract, skin ulcers, eczema and respiratory lung cancer.

Table 2:Concentration of Heavy Metals in the Animal Skin samples

METALS	A	B	C	WHO	USEPA
Fe^{+2}	0.068 ±0.001	0.064 ±0.001	0.067 ±0.001	0.01	0.3
Cd^{+2}	0.001 ±0.003	0.002 ±0.003	0.006 ±0.001	0.003	0.05
Pb^{+2}	0.002 ±0.002	0.001 ±0.002	0.004 ±0.002	0.01	0.015
Cr^{+2}	0.004 ±0.006	0.002 ±0.006	0.004 ±0.006	0.05	0.1
As^{+2}	BDL	BDL	BDL		

BDL– Below Detection Limit

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Result of the concentration of PAHs in the Animal Skin samples

Identification and quantification of PAHs in the collected animal skin samples was carried out to assessthe presence of USEPA priority PAHs and evaluate the health risk associated with their consumption. The result is presented in Table 3.

Polycyclic aromatic hydrocarbons (PAHs) are a group of contaminants produced by burning of carbon-base materials. They can get into food either from the environment or during food processing. Some PAHs are known to cause cancer because they can damage DNA.

Seventeen types of PAH's compounds were detected in the animal skin samples from the three different markets, all exceeded the maximum permissible limits which is 12 ug/kg for carcinogenic PAH's. The level of Naphthalene, Dibenz[a,h]anthracene, and Dibenzo(a,h)pyrene are higher in A compared to B while C has the lowest. Level of Acenaphthylene, Chrysene, and Benzo[a]pyrene was found to be below detection level in all the samples. The level of Acenaphthene, Fluorene, Anthracene, and Benzo[c]phenanthrene are a little higher in C compared to A while B is the lowest. The level of Phenanthrene, 3-Methylcholanthrene, Benzo[ghi]perylene, and Dibenzo(a,i)pyrene was found to be higher in B compared to A while C is the lowest. The level of Fluoranthene, and Benzo[j+k+b]fluoranthene was found to be higher in A compared to C while B is the lowest. The level of Pyrene, Benz[a]anthracene, Indeno[1,2,3-cd]pyrene, was found to be higher in B compared to C while A is the lowest.

The exposure limit for naphthalene as regulated by the National Institute for Occupational Safety and Health (NIOSH) is 10–15 µg/g. Hence, depending on the regularity of consumption and the amount consumed per time, irritation of the eyes, skin irritation, respiratory system discomfort, wheezing, shortness of breath, bronchitis, vomiting, kidney and liver damage could manifest as symptoms of systemic acenaphthylene overload in the consumers.

The results of this findings supports the work of Abdel-Shafy and Mansour, [34] which attributed the increase in concentration of PAHs in animal skin to accidental exposure during grazing of animal to contaminants in the grazing field. According to Adams et al. [15] the materials used in singeing may increase the concentration of PAHs in singed animal skin. PAH's can enter the body through inhalation, ingestion, and contact. Long term health effects of exposure to PAH's may include cataracts, kidney, and liver damage. Polyaromatic hydrocarbons(PAHs) also generates reactive intermediates such quinine, hydroxyl alkyl derivatives, which are not sufficiently polar to be excreted then tend to form covalent addicts with nucleic acid and lead to genetic effects.

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Table 3:Levels of PAHs in the sampled animal skin (µg/kg)

PAH	A	B	C
*Naphthalene	40.0 ±0.5	20.1 ±0.005	16.0 ±0.005
*Acenaphthylene	BDL	BDL	BDL
*Acenaphthene	36.0 ±0.1	35.7 ±0.2	36.2 ±0.1
*Fluorene	16.1 ±0.1	15.8 ±0.2	16.2 ±0.1
*Phenanthrene	4.2 ±0.1	4.3 ±0.1	3.9 ±0.1
*Anthracene	32.2 ±0.001	31.8 ±0.2	32.4 ±0.1
*Fluoranthene	40.2 ±0.5	36.2 ±0.3	36.4 ±0.5
*Pyrene	20.2 ±0.3	20.5 ±0.4	20.3 ±0.2
Benzo[c]phenanthrene	32.3 ±0.4	32.1 ±0.5	32.6 ±0.3
**Benz[a]anthracene	56.2 ±0.5	60.1 ±0.4	56.4 ±0.5
**Chrysene	BDL	BDL	BDL
**Benzo[a]pyrene	BDL	BDL	BDL
Benzo[e]pyrene	55.8 ±0.6	56.2 ±0.5	56.4 ±0.3
**Benzo[j+k+b]fluoranthene	72.3 ±0.5	71.8 ±0.6	72.0 ±0.3
3-Methylcholanthrene	40.3 ±0.5	72.0 ±0.5	36.2 ±0.5
**Indeno[1,2,3-cd]pyrene	40.1 ±0.1	44.2 ±0.3	40.3 ±0.3
**Dibenz[a,h]anthracene	68.2 ±0.4	68.1 ±0.3	64.2 ±0.2
**Benzo[ghi]perylene	39.8 ±0.2	40.0 ±0.1	36.4 ±0.3
Dibenzo(a,h)pyrene	16.3 ±0.1	16.2 ±0.3	15.8 ±0.1
Dibenzo(a,i)pyrene	28.4 ±0.1	32.1 ±0.2	28.3 ±0.1
PAH4	128.5	131.9	128.4

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Results of PAHs in this study are lower than the level reported by Okareh et al. [20] but, higher than the results of Oyekunle et al. [35] for PAHs in beef sausage.

Carcinogernic risk assessment of polycyclic aromatic hydrocarbons in animal skin

The cancer risk due to dietary exposure to PAHs was assessed using the individual PAH carcinogenic potencies(B(A)Pteq), the carcinogenic toxic equivalents (TEQ) and PAH4 index [35,36,37]. The Carcinogenic potencies of individual PAHs B(A)Pteqwas evaluated by multiplying the PAH concentration in the sample by the individual toxicity equivalency factor (TEF) (Eq. (3). TheTEF is an estimate of the relative toxicity of individual PAH fraction compared to benzo(a)pyrene.

Carcinogenic potencies of individual PAHs (B(A)Pteq) = $C_i \times TEF_i$ (3)

The carcinogenic toxic equivalent (TEQs) was then obtained by summing the carcinogenic potencies of individual PAHs as indicated in Equation 4.

Carcinogenic toxic equivalents(TEQs) = $\sum$(B(A)Pteq) (4)

The result of individual PAH carcinogenic potencies (B(A)Pteq) and carcinogenic toxic equivalents (TEQ) is presented in Table 4. These values are higher than the values reported by [35, 36].

The PAH4 index was assessed in this study based on the review by the Contaminants in the Food Chain (CONTAM) Panel, (2008). PAH4 is a more suitable indicator of PAHs in Food relating to occurrence and toxicity of PAHs in food,

PAH4 Index (PAH4) = ————(B[a]A + Chr + B[b]FL + B[a]P) (2)

Table 4: Results of Carcinogenic potencies (B(A)Pteq)and Carcinogenic Toxic Equivalent (TEQs) of the animal skin samples

					B(A)Pteq			
PAHs	Conc.	A (µg/kg)	B (µg/kg)	C (µg/kg)	A	B	C	TEQ (µg/kg)
Naphthalene	0.00	40.00	20.10	16.00	0.04	0.02	0.02	0.08
Acenaphthene	0.00	36.00	35.70	36.20	0.04	0.04	0.04	0.11
Fluorene	0.00	16.10	15.80	16.20	0.02	0.02	0.02	0.05
Phenanthrene	0.00	4.20	4.30	3.90	0.00	0.00	0.00	0.01
Anthracene	0.10	32.20	31.80	32.40	3.22	3.18	3.24	9.64

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Fluoranthene	0.00	40.20	36.20	36.40	0.04	0.04	0.04	0.11
Pyrene	0.00	20.20	20.50	20.30	0.02	0.02	0.02	0.06
Benz[a]anthracene	0.10	56.20	60.10	56.40	5.62	6.01	5.64	17.27
Benzo[e]pyrene	0.10	55.80	56.20	56.40	5.58	5.62	5.64	16.84
Benzo[j+k+b]fluoranthene	0.01	72.30	71.80	72.00	0.72	0.72	0.72	2.16
Indeno[1,2,3-cd]pyrene	0.10	40.10	44.20	40.30	4.01	4.42	4.03	12.46
Dibenz[a,h]anthracene	5.00	68.20	68.10	64.20	341.00	340.50	321.00	1002.50
Benzo[ghi]perylene	0.01	39.80	40.00	36.40	0.40	0.40	0.36	1.16

The maximum level for the sum of PAH4 has been 12.00 μg/kg of a product (European Commission, 2002). The sum of PAH4 in samples A, B and C (μg/kg) is 128.5, 131.9 and 128.4 respectively. PAH4 in all the animal skin samples from the three sites are higher than the EFSA recommended maximum level. indicates their consumption should be discouraged because most of these Polyaromatic hydrocarbons are carcinogenic in nature and excessive consumption can lead to death. It is therefore advised that cow skin be discouraged, and more awareness created so that people will know the dangers to health posed by this harmful practice.

CONCLUSION

This study determined the nutritional value of Cowskin, and the result of the proximate analysis shows that Cowskin samples analyzed had a very low protein content which is erroneously believe to be its major nutritional value. The results of this study reveal the presence of heavy metals in the animal skins though, below the maximum permissible limit of WHO and USEPA. Also 17 PAH's were detected in the cow skin samples and all were above the maximum permissible limits which

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