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POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN THE FRUITS OF *Cucumis sativus* FROM TWO MARKETS WITHIN ABA METROPOLIS

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ABSTRACT: Polycyclic aromatic hydrocarbons (PAHs) were investigated in the fruits of *Cucumis sativus* obtained from Ahiaohuru and Ehere markets within Aba metropolis. *C. sativus* obtained from each of these markets was divided into three portions. The first portion was not washed (UW), the second portion was washed with tap water (WTW) while the last portion was washed with an aqueous solution of sodium chloride (WSS) before drying, milling and extraction procedures were applied. Quantification of the PAHs was done using a Gas Chromatography/Mass Spectrometry (GC/MS) technique. Eighteen PAHs were analyzed and *C. sativus* samples from Ahiaohuru market gave a total PAHs content of 30.45, 25.65 and 2.97 mg kg^{-1} for UW, WTW and WSS, respectively. The samples from Ehere market gave 29.88, 18.82 and 3.06 mg kg^{-1} for UW, WTW and WSS, respectively. The results obtained from this research indicates that the level of PAHs content in the studied samples decreased when washed with tap water and further decreased significantly when washed with aqueous solution of sodium chloride. From the risk assessment of ΣPAH4 (the sum of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) in the sample, there was no risk associated with the consumption of the fruity samples. It is however, advisable to wash fruity vegetables with a salt solution as it would reduce the presence of PAHs in them better than washing with tap water alone.

Keywords: PAHs; *C. sativus*; GC/MS; Fruity vegetables; Risk Assessment

INTRODUCTION

Fruity vegetables provide essential nutrients in man's diet. Besides, plants in general still provide humongous and invaluable phytochemicals for a wide and myriads of treatments against diseases and infections [1]. The consumption of fruity vegetables has been associated with better cardiovascular health, reduced risk of some cancers, better mental health and greater longevity [2, 3]. One of the fruity vegetables eaten widely in Nigeria is *Cucumis sativus*. *Cucumis sativus* commonly known as cucumber belongs to the *Cucurbitaceae* family [4].

It is an annual climber growing up to 2 m. The fruit is roughly cylindrical, elongated with tapered ends, and may be as large as 60 cm long and 10 cm in diameter [5]. The cucumber is a common ingredient of salads, being valued mainly for its crisp texture and juiciness. However, it is very watery, with little flavour. It is widely consumed fresh in salads or fermented (pickles) or as a cooked vegetable [6]. *C. sativus* has been reported to possess antimicrobial, antifungal, antioxidant and hypoglycaemic activities [7]. *C. sativus* is used for jaundice, bleeding disorders and

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anuria while its seeds are highly nourishing [5].

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic xenobiotic fused-ring aromatic compounds consisting of hydrocarbon molecules of two or more fused benzene and/or pentacyclic rings in linear, angular or cluster formation [8,9]. PAHs are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials like coal, oil, petrol and wood [10]. In other words, PAHs are formed whenever organic substances are exposed to high temperatures under low oxygen or no oxygen conditions [10,11]. PAHs are usually found as contaminants in food resulting from deposition of airborne particulates on their exposed surfaces, environmental pollution and food processing steps [8,12,13].

This research work aims at the quantification of PAHs on the surface of *C. sativus* obtained from two markets within Aba metropolis in relation to the method of washing before analyses. It is a common practice to wash fruits before consuming them. While most people wash with tap water, others may wash with a common salt solution [14]. Hence this research probes the PAHs contents of the fruit samples in order to generate a scientific evidence for valid recommendations. The results of the aforesaid analyses are herein reported.

MATERIALS AND METHODS

Sample Collection

The fruits of *C. sativus* (cucumber) were obtained from Ahiaohuru and Ehere markets in the month of December, 2021. The samples were identified and authenticated at the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria.

Sample Preparation

C. sativus from each of the two markets was divided into three portions of about 200 g per portion. The first portion was not washed prior to drying. The second portion was washed with tap water to remove dirt and impurities. The third portion was washed with 0.17 mol dm^{-3} of sodium chloride solution and finally rinsed with tap water. The various portions were sliced thinly with a clean kitchen knife and were oven-dried at 65$^{\circ}C$ for 144 h. The dried samples were pulverized to fine and smooth particles with a wooden mortar and pestle and were stored in air-tight containers prior to analysis.

Extraction of Samples for the PAHs Determination

In triplicates, l00 mg of each sample was weighed into clean extraction containers. 10 ml of extraction solvent (dichloromethane) was added into each and

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mixed thoroughly and allowed to settle. The mixture was carefully filtered into clean solvent-rinsed extraction bottles, using filter paper fitted to Buchner funnels. The extractions were concentrated to 2 ml and were cleaned up using column chromatography.

Clean-up/Separation Technique

A chromatographic column of length 280 mm and diameter of 15 mm was used. Cotton wool was used to plug the outlet of the column to about 2 cm. A slurry of 2 g activated silica gel in 10 ml dichloromethane (DCM) was prepared and introduced into the chromatographic column. Care was taken while tapping the column (with pencil fitted with rubber stopper) constantly and gently on the sides during the introduction of the slurry which promoted even settling and mixing and gave an evenly packed column free of air bubbles. To the top of the column was added 0.5 cm of sodium sulphate and DCM was cycled through the column several times to ensure that the setting was complete and that the column was firmly packed. The last cycle was stopped at the point when the liquid was just above the sulphate layer. Immediately, 1 ml of the extracted sample was transferred into the column and the extraction bottle was rinsed with 1 ml DCM and was also added to the column. Elution started and the eluant was collected with a 50 ml beaker. Just before the exposure of the sodium sulphate layer to air, hexane was added to the column in 1-2 ml increments. A measured volume of 8-10 ml of the eluant was collected and labeled. The volumes collected were concentrated and transferred into labeled glass vials with rubber caps for GC/MS analysis.

GC/MS Analysis

An Agilent 6890N gas chromatography equipped with an auto-sampler connected to an Agilent mass spectrophotometric detector was used. 1 µl of sample extract was injected in the pulsed spitless mode onto a 30 m x 0.25 mm id DB 5MS coated fused silica column with a film thickness of 0.15 µm. Helium gas was used as a carrier gas and the column head pressure maintained at 20 psi to give a constant of 1 ml/min. Other operating conditions were preset. The column temperature initially held at 55°C for 0.4 min, was increased to 200°C at a rate of 25°C /mins, then to 280°C at a rate of 8°C/mins and to final temperature of 300°C at a rate of 25°C/mins, held for 2 mins. The identification time was based on retention time since each of the PAHs has its separate retention time in the column. The PAHs components with lower retention time were eluted before the ones with higher retention time. The column was calibrated with PAH standards supplied by instrument manufacturer. A calibration curve was obtained by analyzing each of the standard PAHs solutions prepared on the GC/MS. The target PAH compound/internal standard peak heights were plotted against the PAH concentration to obtain a linear graph Y= mx + b, with an intercept (b) on the y-axis. All

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the samples were analyzed for eighteen PAH congeners: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, dibeno[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, 2-methylnaphthalene, naphthalene, phenanthrene, pyrene and 1,2,3-trimethylbenzene.

Risk Assessment of PAHs

Risk assessment is the process that evaluates the potential health effects on humans from contaminant doses received through one or more exposure pathways [15]. Dietary and carcinogenic methods were used in this study to assess the potential risks posed by PAHs to human consumers of the fruity vegetables.

Dietary Exposure Assessment

Daily dietary exposure level by the populace to PAH4 was calculated with the formula [16].

$$E = C \times IR$$

……..……..eq. 1

In equation 1, E = daily dietary ΣPAH4 exposure level (ng/day); C= PAH4 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) concentration in vegetables (ng/g); IR = daily ingestion rate vegetables which was estimated to be 65 g/day [17].

Cancer Risk Assessment

The Margin of Exposure (MOE) approach was used for the characterization of the risk posed to human by exposure to PAHs which can cause cancer or damage genetic material. It is a ratio which for a specific population, assesses the dose at which a small but measurable negative effect is initially noticed and the level of exposure to the target substance [18].

$$MOE = \frac{BMDL_{10} \times BW}{E}$$

……..eq. 2

In equation 2, $BMDL_{10}$ (benchmark dose lower confidence limit 10 %) is an estimate of the lowest dose, which is 95 % certain to cause no more than a 10 % cancer incidence in animals. It was calculated as 0.34 mg/kg b.w./day for ΣPAH4 [19]. BW is the average body weight which is set as 60.7 kg for adults [20]. MOE $\geq$ 10,000 is assumed to be of low risk concern from a public health standpoint [21].

RESULTS AND DISCUSSION

The dry weight PAHs concentrations in the sample of *C. sativus* obtained from two market locations are shown in Tables 1 and 2 below.

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Table 1: PAHs concentrations (mg kg^{-1}) in *C. sativus* fruit samples from Ahiaohuru Market

Contaminants	Unwashed	Washed with tap water	Washed with salt water
1,2,3-Trimethylbenzene	3.30±0.03	4.06±0.03	0.33±0.01
Naphthalene	2.94±0.02	3.11±0.03	0.30±0.01
2-Methylnaphthalene	2.99±0.02	1.44±0.01	0.43±0.02
Acenaphthylene	1.79±0.01	2.09±0.02	0.23±0.01
Acenaphthene	2.31±0.03	0.94±0.04	0.05±0.005
Fluorene	3.13 ±0.02	1.12±0.03	0.08±0.01
Anthracene	1.23±0.01	1.40±0.01	0.18±0.06
Phenanthrene	3.32±0.03	3.11±0.02	0.20±0.01
Fluoranthene	2.33±0.02	0.88±0.02	0.18±0.01
Pyrene	0.94±0.02	0.98±0.01	0.19±0.03
Benzo[a]anthracene	0.96±0.04	0.40±0.01	0.12±0.01
Chrysene	0.39±0.01	0.34±0.01	0.09±0.009
Benzo[b]fluoranthene	0.63±0.02	1.79±0.01	0.08±0.01
Benzo[k]fluoranthene	0.41±0.08	0.81±0.03	0.07±0.009
Benzo[a]pyrene	0.59±0.01	0.80±0.05	0.09±0.007
Diben[a,h]anthracene	1.23±0.01	0.89±0.03	0.10±0.06
Indeno[1,2,3-cd]pyrene	0.36±0.03	0.70±0.04	0.13±0.02
Benzo[g,h,i]perylene	1.60±0.01	0.79±0.01	0.12±0.01
$\sum PAH4$	**2.57**	**3.33**	**0.38**
$\sum PAH18$	**30.45**	**25.65**	**2.97**

Values are means± standard deviation of triplicate determinations. ND means not detected

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Table 2: PAHs concentrations (mg kg^{-1}) in *C. sativus* fruit samples from Ehere Market

Contaminants	Unwashed	Washed with tap water	Washed with salt water
1,2,3-Trimethylbenzene	6.16±0.02	4.13±0.01	0.33±0.01
Naphthalene	5.07±0.02	3.08±0.01	0.38±0.03
2-Methylnaphthalene	2.04±0.02	2.75±0.01	0.29±0.05
Acenaphthylene	2.10±0.04	2.28±0.02	0.09±0.01
Acenaphthene	3.01±0.04	1.05±0.01	0.16±0.01
Fluorene	1.01 ±0.02	0.21±0.02	0.06±0.01
Anthracene	0.87±0.02	0.45±0.01	0.20±0.03
Phenanthrene	0.99±0.03	0.41±0.02	0.27±0.02
Fluoranthene	0.89±0.03	0.39±0.05	0.21±0.01
Pyrene	0.97±0.02	0.52±0.02	ND
Benzo[a]anthracene	0.97±0.03	0.49±0.01	ND
Chrysene	0.83±0.01	0.33±0.01	0.29±0.01
Benzo[b]fluoranthene	0.68±0.02	0.61±0.01	0.14±0.01
Benzo[k]fluoranthene	0.72±0.02	0.48±0.05	0.32±0.03
Benzo[a]pyrene	0.88±0.03	0.35±0.02	0.32±0.02
Diben[a,h]anthracene	0.57±0.01	0.49±0.01	ND
Indeno[1,2,3-cd]pyrene	1.09±0.01	0.28±0.01	ND
Benzo[g,h,i]perylene	1.03±0.02	0.52±0.03	ND
$\sum PAH4$	**3.36**	**1.78**	**0.75**
$\sum PAH18$	**29.88**	**18.82**	**3.06**

Values are means ± standard deviation of triplicate determinations. ND means not detected

The PAHs concentrations (mg kg^{-1}) in *C. sativus* samples from Ahiaohuru market are shown in Table 1 while that from Ehere market are shown in Table 2. Observation shows that the unwashed portions of the samples from the two markets registered the highest total PAHs contents ($\sum$PAH18) (30.45; 29.88 mg kg^{-1}) followed by the portions washed with tap water (25.65; 18.82 mg kg^{-1}). There were significant reductions in the PAHs contents of the third portions that were washed with aqueous solution of

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sodium chloride i.e. (2.97; 3.06 mg kg^{-1}). Also, $\sum PAH4$ which is the sum of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene concentrations in the analyzed samples gave (2.57; 3.36 mg kg^{-1}), (3.33; 1.78 mg kg^{-1}) and (0.38; 0.75 mg kg^{-1}) for the UW, WTW and WSS from Ahiaohuru and Ehere markets, respectively. Osu and Okoro [22] reported that the values of PAHs detected in *Vernonia amygdalina*, *Telfera occidendalis* and *Amaranthus spinosus* grown in a garden located at an automobile mechanic workshop in Choba district area of Port Harcourt metropolis in Nigeria, ranged from 32.50 ± 0.10 to 54.67 ± 0.10 µg kg^{-1} (i.e. 0.0325 to 0.05467 mg kg^{-1}). The values obtained in this study are higher than what they reported. Igwe *et al.* [23] reported values of 11.94, 7.17 and 0.27 mg kg^{-1} as the total PAHs content ($\sum PAH18$) in *Talinum triangulare* from Afule, Ahiaohuru and Ohanku markets in Aba metropolis, respectively. The values of total PAHs content ($\sum PAH18$) reported in this research work for UW, WTW and WSS are much higher. They also reported values of 5.70 and 9.03 mg kg^{-1} as total PAHs contents for *P. guineense* from Afule and Ohanku markets respectively. These values are much lower than the values reported herein for UW and WTW.

Table 3: Risk assessment of ΣPAH4 in the fruit sample of *C. sativus*

Risk Parameters	**Ahiaohuru Market**			**Ehere Market**		
	UW	WTW	WSS	UN	WTW	WSS
E (ng/day)	167.05	216.45	24.70	218.40	115.70	48.75
MOE	123544	95348	835547	94496	178375	423344

E = Dietary Exposure; MOE = Margin of Exposure, UW = Unwashed sample, WTW = Washed with tap water; WSS = Washed with salt solution

Table 3 shows the risk assessment for ΣPAH4 found in the samples. PAHs risk assessments are based on benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene concentrations in the analyzed samples which is a process that evaluates the potential health effects on humans from contaminant doses received through one or more exposure pathways [15]. Benzo[a]pyrene and $\sum PAH4$ are used by different authorities as indicators or markers for the occurrence of PAHs in food. However, the European Food Safety Agency has determined that $\sum PAH4$ is a better indicator than benzo[a]pyrene [24]. It is noteworthy that there are no regulatory limits for benzo[a]pyrene and total PAHs in vegetables [18]. The daily dietary ΣPAH4 exposure level (ng/day) ranged from 24.70 to 216.45 for samples from Ahiaohuru market while that from Ehere market ranged from 48.75 to 218.40. Igwe *et al.*

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[23] reported values of daily dietary exposure levels that ranged from 0.00 to 259.35 ng/day for *Talinum triangulare* and *Piper guineense* obtained from farms near markets within Aba metropolis. The use of the $BMDL_{10}$ value of 0.34 mg/kg bw/day set by EFSA [19], a daily vegetable consumption of 65 g per person and an adult body weight of 60.7 kg resulted in MOE values higher than 10,000 in all the samples containing PAH4. MOE $\geq$ 10,000 is assumed to be of low risk concern from a public health standpoint [21]. The values of MOE in Table 3 are all more than 10000. It then means that there are no health risks associated with the consumption of *C. sativus* obtained from Ahiaohuru and Ehere markets within the period of this research.

CONCLUSION

This research investigated the PAHs contents of *C. sativus* obtained from Ahiaohuru and Ehere markets within Aba metropolis with respect to two different washing methods. The samples washed with an aqueous solution of sodium chloride contained the lowest PAHs in contrast with the ones washed with tap water. The risk assessment of the PAHs showed that the consumption of the *C. sativus* samples is not associated with any risk. However, it is advisable and recommended based on the outcome of this research to wash fruity vegetables with a common salt solution before rinsing with a tap water as this would reduce the PAHs deposited on them to the barest minimum.

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