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Article

Persistent Organochlorine Pesticide Residues in Some Selected Cocoa Beverages in Nigeria

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Abstract: This study evaluates the quality of the cocoa beverages produced in Nigeria with respect to the occurrence and levels of organochlorine pesticides OCPs residues in order to ascertain the potential health risks to the general public. Seven cocoa-based beverages were analysed for 17 OCP residues using gas chromatography coupled with an Electron Captured Detector (GC-ECD) after extraction and silica-gel clean-up. The study reveals the presence of ten OCP residues in the cocoa beverages, with a concentration range from not detected ND—0.256 mg/kg, while α-BHC, β-BHC, methoxychlor, p,p′-DDE, dieldrin, endrin aldehyde, and endosulfan sulphate were not detected in any of the analysed samples. The contamination pattern of OCPs in the beverages was in the following order: Ovaltine > Milo > Cadbury-choco > Bournvita > Cowbell-coffee > Richoco > Oluji, with p,p′-DDT being the most frequently found pesticide. Heptachlor and endosulfan II showeda residual level above the European Union (EU) Maximum Residual Limits (MRLs) in only one sample.

Keywords: organochlorine; cocoa beans; beverages; pesticides residues; gas chromatograph

1. Introduction

Cocoa is of vital importance to the economies of producing countries in West Africa, namely, Ivory Coast, Ghana, Nigeria, Cameroun, and Togo [1]. Cocoa contributes over 26% of the non-oil export component of Nigeria's Gross Domestic Product (GDP) [2,3]. Cocoa beverages, which are largely consumed as dietary supplements [4,5], are produced from cocoa beans that have regularly received pesticides with the aim of improving cocoa farms' income and diversification of foreign exchange earnings by increasing cocoa production. The nutritional parameters of cocoa are determined largely by the chemical composition of the material [6]. The quality parameters of different consumed Nigeria cocoa-based beverages have been reported by Olaofe et al. [7].

Cocoa plantations are often susceptible to varieties of pests and diseases, which some reports have estimated to account for global production loss of 30% to 40% [8].The excessive use of these pesticides, coupled with their recalcitrant nature, has resulted in the presence of pesticide residues in cocoa farm products [9]. Most pesticides, particularly organochlorines (OCs), are toxic to biological organisms, due to their high lipophilic properties [10], their resistance to chemical and biological degradation [11],their ability to adsorb onto particulate matter due to low water solubility [12], and their ability to bioaccumulate and biomagnify [13,14].

Given the potential risk that pesticides pose to public health, the use of pesticides in cocoa production is subject to constant monitoring. In Nigeria, levels of OCPs in cocoa beans have been reported by [15,16]. However, pesticides are poorly monitored in Nigerian foods, particularly dietary items. Therefore, the objective of this study was to evaluate the quality of cocoa-based beverages produced in Nigeria with respect to occurrence and levels of OCP residues in order to ascertain whether the levels were above the permissible limits set by the EU and FAO/WHO.

2. Materials and Methods

2.1. Chemicals and Reagents Used

The reagents used were of spectral purity. They included GC-grade n-hexane (Uiltrafine, Marlborough House, London, UK), acetone (GFS Chemicals, Columbus, OH, USA), silica gel (Labtech Chemicals, Lewes, Brighton) and anhydrous Na_2SO_4 (Merck, Darmstadt, Germany). These reagents were procured through various representatives of manufacturing companies in Nigeria. The silica gel and anhydrous sodium sulphate were heated in an oven at 120 °C for 12 h to ensure that no adsorbed water could be found.

2.2. Sampling

The samples (cocoa beverages) were purchased at a Supermarket in Ado-Ekiti, South-Western Nigeria, and the selection was based on the availability of the samples at the point of purchase. Two samples were collected for each brand of cocoa beverage, making a total of 14 samples. The samples were purchased from the same location, and kept in a cool dry place for about five days prior to extraction and analysis. The samples were in powder form; samples with some solid part were ground to a powdery form with the aid of an agate mortar and pestle, so as to have a homogenous sample. The samples were stored in the refrigerator at 4 °C prior to analysis. Sampling of the beverages was done in March 2016.

2.3. Pesticide Extraction

For pesticide extraction and clean-up of the cocoa beverages, the EPA 3550C method was used, as described by [17]. Approximately 20 g of each ground sample and 20 g of anhydrous Na_2SO_4 was mixed together in a pre-cleaned 250 mL conical flask. About 50 mL of acetone and n-hexane (1:1 v/v) were added to the sample, and the mixture were sonicated in a high frequency ultrasonic bath for 10–15 min. The extraction process was repeated with an additional 50 mL (acetone and n-hexane mixture), which was then sonicated, cooled and decanted into the same round bottom flask. The resulting extract was concentrated to 2 mL using a rotary evaporator at 40 °C. The extract was re-dissolved in 5 mL n-hexane, and again concentrated to 2 mL in a rotary evaporator.

2.4. Clean-Up Procedure

A column of about 15 cm (length) × 1 cm (internal diameter) was packed with glass wool and later with 2 g of activated silica gel (Silica gel 60 F_{254}). About 1 g anhydrous Na_2SO_4 was placed at the top of the column to absorb water. Pre-elution was done with 15 mL n-hexane prior to the clean-up. The extract was run through the column and eluted with 20 mL n-hexane and diethyl ether (1:1 v/v). The eluate was concentrated to dryness on the rotary evaporator, and then recovered into 2 mL n-hexane. The final extract was later transferred into GC vials for GC analysis.

2.5. Identification, Quantification and Quality Control

The limits of detection (LOD) were determined at a signal-to-noise ratio (S/N) of 3 for each pesticide. A detectable ion should produce a signal that is at least three times the baseline noise; that is, signal-to-noise ratio = 3. The limit of quantification (LOQ) was obtained at a signal-to-noise ratio (S/N) of 10 for each pesticide by GC-ECD. The LOD and LOQ ranged from 0.001 to 0.005 mg/kg, and from 0.003 to 0.010 mg/kg, respectively. To determine the validity of the methodology, a standard addition method was employed, in which a known amount of pesticide was added to the samples, and then analysed for the total amount of OCP. Samples were spiked with mixed OCP standard solutions (1, 2, 5 μg/L). The spiked samples were allowed to stand for several hours, and were then extracted, cleaned, and analysed, as described above. Recovery and precision (expressed as relative standard deviation) were calculated for three replicated samples, and the data are presented in Table 1.

Standard solutions of OCPs were run in GC-(ECD) under set chromatographic conditions and mean peak areas were plotted against concentrations to obtain the calibration curves of individual pesticides. Under set chromatographic conditions, a standard calibration curve was prepared for each OCP. The signature retention time for each OCP was used as a confirmatory indicator. Linearity was determined by plotting the calibration curve with the standard solution in n-hexane containing four different concentrations (0.1, 0.25, 0.5, 1.0 ng/µL).

Table 1. Mean percent recovery for the pesticides.

OCPs	Percent Recovered
Aldrin	87.1 ± 2.9
δ-BHC	81.7 ± 3.5
γ-BHC	89.3 ± 4.4
p,p′-DDT	91.6 ± 5.2
Heptachlor	89.2 ± 4.1
Heptachlor-epoxide	91.4 ± 3.6
Endosulfan I	84.7 ± 5.8
Endosulfan II	92.5 ± 8.6

2.6. Gas Chromatographic Condition

The gas chromatography conditions for the analysis were as follows: GC model: Agilent 7890A coupled with electron capture detector (GC-ECD); injector and detector temperature were 250 °C and 390 °C; the purge activation time was 30 s; inlet mode: splitless with flow rate of 2 mL/min; carrier gas: helium; make-up gas: nitrogen; inlet temperature: 250 °C; column type: DB-17 fused silica capillary column; column dimension: 30 m × 250 µm × 0.25 µm film thickness; oven condition: initial temperature at 150 °C and increase to 280 °C at 6 °C/min. The total run time was 21.667 min.

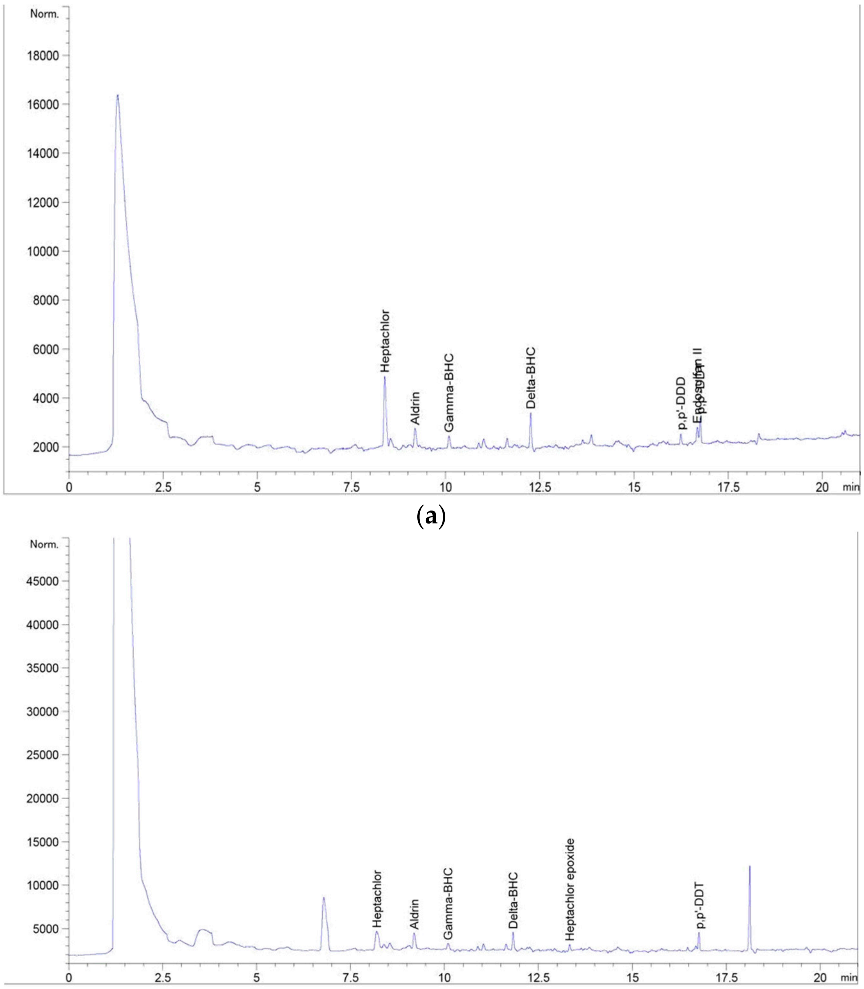
2.7. Statistical Analysis

For the statistical analysis, one-way analysis of variance (ANOVA) was used to test for the significant difference between the pesticide residues detected in the cocoa beverages. Differences were considered significant at $p > 0.05$.

3. Results and Discussion

Figure 1a,b shows representative chromatograms of the samples. No interference peaks were obtained for the blank sample chromatogram at the same retention time as the target compounds. The mean recovery values for the spiked samples are shown in Table 1. The percent recovered range was between 81.7% (δ-BHC) and 92.5% (endosulfan II). The mean percentages for recoveries were within the 70–110% acceptable range for recovery, as stipulated by EU guidelines for evaluating accuracy and precision of a method [18], and thus show that the procedure employed in this study is reproducible, efficient, and reliable for the analysis of OCPs.

The mean levels of chlorinated benzenes in the selected cocoa beverages are shown in Table 2. The concentration of benzene hexachloride BHCs ranges from ND—0.005 mg/kg, while the mean concentration ranges from 0.001 (γ-BHC)—0.004 (δ-BHC) mg/kg. Cowbell, Ovaltine, Oluji and Richoco exhibited no detection of any BHCs; Milo and Bournvita only showed the presence of γ-BHC and δ-BHC, while Cadbury-choco had only δ-BHC. The presence of these pesticides shows that cocoa farmers in Nigeria have actively used technical BHC (lindane), which is the only isomer of BHC with pesticidal activity. Presence of BHCs has been reported in Nigeria cocoa farms [9,19],which suggests the previous use of technical BHC in Nigerian cocoa production. The results of lindane in this study were within the levels reported by Frimpong et al. [20] and Okoffo et al. [21], while Oyekunle et al. [16] showed higher levels. The measured concentrations of lindane were far below the European Union (EU) Maximum Residue Limits (MRLs) of 1.0 mg/kg.



(**a**)

(**b**)

Figure 1. (**a**,**b**) Representative chromatograms of the samples.

Table 2. Mean concentration (mg/kg) of benzene hexachloride in the cocoa beverages.

OCPs	CWBL	CADC	MILO	OVAL	OLUJ	BOUN	RICH
α-BHC	ND	ND	ND	ND	ND	ND	ND
β-BHC	ND	ND	ND	ND	ND	ND	ND
γ-BHC	ND	ND	0.002 ± 0.002	ND	ND	0.001 ± 0.001	ND
δ-BHC	ND	0.004 ± 0.001	0.003 ± 0.002	ND	ND	0.003 ± 0.001	ND

CWBL = Cowbell-coffee; CADC = Cadbury-choco; MILO = Milo; OVAL = Ovaltine; OLUJ = Oluji; BOUN = Bournvita; RICH = Richoco; ND = Not detected.

The mean concentrations of dichlorodiphenylethanes in the selected beverages are shown in Table 3. The dichlorodiphenylethanes level range from ND—0.118 mg/kg. Methoxychlor and p,p′-DDE showed not detected in all the samples, while p,p′-DDT was present in all the samples with a mean concentration range of 0.001 mg/kg (Richoco) to 0.118 mg/kg (Ovaltine). p,p′-DDD was only detected in Ovaltine.

The p,p′-DDT level was comparably similar to those reported by [20], but lower than those reported by [16]. The DDT (p,p′-DDE, p,p′-DDD and p,p′-DDT) concentrations from the present study were below the EU-MRL of 0.50 mg/kg in food. Statistical analysis showed no significant difference ($p > 0.05$) in the DDT levels.

Table 3. Mean concentration (mg/kg) of dichlorodiphenylethene in the cocoa beverages.

OCPs	CWBL	CADC	MILO	OVAL	OLUJ	BOUN	RICH
p,p′-DDE	ND	ND	ND	ND	ND	ND	ND
p,p′-DDD	ND	ND	ND	0.003 ± 0.002	ND	ND	ND
p,p′-DDT	0.003 ± 0.002	0.004 ± 0.002	0.008 ± 0.001	0.118 ± 0.000	0.003 ± 0.001	0.004 ± 0.002	0.001 ± 0.00
Methoxychlor	ND	ND	ND	ND	ND	ND	ND

CWBL = Cowbell-coffee; CADC = Cadbury-choco; MILO = Milo; OVAL = Ovaltine; OLUJ = Oluji; BOUN = Bournvita; RICH = Richoco; ND = Not detected.

Table 4 present the levels of cyclodienes (mg/kg) in the selected cocoa beverages. Six cyclodiene pesticides were detected in the cocoa beverages, with a concentration range of ND—0.256 mg/kg, while dieldrin, endrin aldehyde and endosulfan sulphate showed not detected in all the cocoa beverages. The concentration (mg/kg) of cyclodienes in Cowbell coffee, Cadbury-choco, Milo, Ovaltine, Richoco and Bournvita ranged from ND—0.011, ND—0.024, ND—0.022, ND—0.256, ND—0.002 and ND—0.015, respectively, while Oluji showed no presence of cyclodienes. The concentration pattern of the cyclodienes was in the following order: Ovaltine > Cadbury-choco > Milo > Bournvita > Cowbell-coffee > Richoco. Aldrin was the most predominant cyclodienes in the cocoa beverages, while endrin showed the least. The predominance of aldrin is probably be due to its active usage by Nigerian cocoa farmers and, most importantly, from where the cocoa beans were bought by the companies. The aldrin level reported in this study was lower than 0.01 mg/kg reported by [20] for aldrin in cocoa beans from Ghana. The aldrin residue concentrations in the present study were below the EU MRLs of 0.05 mg/kg.

Table 4. Mean concentration (mg/kg) of cyclodienes in the cocoa beverages.

OCPs	CWBL	CADC	MILO	OVAL	OLUJ	BOUN	RICH
Heptachlor	ND	ND	0.006 ± 0.014	0.029 ± 0.076	ND	0.008 ± 0.020	ND
Hept.-epoxide	ND	0.003 ± 0.005	0.001 ± 0.001	0.002 ± 0.003	ND	ND	ND
Aldrin	ND	0.001 ± 0.001	0.005 ± 0.009	0.023 ± 0.064	ND	0.002 ± 0.003	0.001 ± 0.001
Dieldrin	ND	ND	ND	ND	ND	ND	ND
Endrin	ND	0.006 ± 0.001	ND	ND	ND	ND	ND
Endrinaldehy	ND	ND	ND	ND	ND	ND	ND
Endosulfan I	0.001 ± 0.001	0.002 ± 0.004	ND	0.021 ± 0.054	ND	ND	ND
Endosulfan II	0.006 ± 0.014	0.012 ± 0.032	ND	0.129 ± 0.340	ND	0.005 ± 0.009	ND
Endosul-sulp	ND	ND	ND	ND	ND	ND	ND

CWBL = Cowbell-coffee; CADC = Cadbury-choco; MILO = Milo; OVAL = Ovaltine; OLUJ = Oluji; BOUN = Bournvita; RICH = Richoco; ND = Not detected.

The heptachlor and heptachlor-epoxide concentrations ranged from ND—0.056 mg/kg with just one sample above the EU MRLs, at 0.02 mg/kg of heptachlor in food samples. This also shows that cocoa farmers in Nigeria have used heptachlor in cocoa production. The heptachlor and heptachlor-epoxide in this study were lower than the mean values of 1.20–1.43 and 2.17–2.80 µg/g reported by [16] in cocoa beans from Ondo and Ile-Ife in Nigeria.

For endrin and endrin aldehyde, only Cadbury-choco showed the presence of endrin, while none of the beverages contained endrin aldehyde. The level of endrin (0.006 mg/kg) was lower than what [16,20] was reported in cocoa beans from Ghana and Nigeria. Frimpong et al. [20] reported that 29 out of 45 (66%) of cocoa beans from Ghana contained endrin, while [16] reported mean values of 0.37 and 6.81 mg/kg. The endrin level in this study was lower than the 0.01 mg/kg listed in the EU MRLs for endrin in food samples.

The endosulfan concentration ranged from ND—0.256 mg/kg with regard to the presence of endosulfan I and endosulfan II, while endosulfan-sulphate was not detected in any of the samples. The level of endosulfan in this study was significantly lower than the 6.83–25.9 mg/kg and 7.42–28.08 mg/kg in Ile-Ife and Ondo, respectively, reported for endosulfans in [16], while those reported in [20] were similar to the present study. Frimpong et al. [20], in their study, reported that 28 out of 45 (64%) cocoa beans from Ghana contained alpha-endosulfan, while beta-endosulfan was detected in 23 out of 45 (52%) samples. The residue levels for the endosulfans in this study were lower than the EU MRLs of 0.10 mg/kg, except in one sample of Ovaltine.

4. Conclusions

The study showed the presence of OCPs with varying concentrations in the beverages, with Ovaltine recording the highest range of pesticide residue. The research generally showed very low levels of OCPs in the cocoa beverages, and thus suggests that they are safe for human consumption. The study therefore recommends constant monitoring of persistent organic pesticides in cocoa beans before being processed for beverages, so as to protect consumers from health-related risks.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

ANOVA	Analysis of variance
BHC	Benzene hexachloride
ECD	Electron capture detector
EU	European Union
GC	Gas chromatography
LOD	Limit of detection
LOC	Limit of quantification
MRLs	Maximum residue limits
ND	Not detected
OCPs	Organochlorine pesticide residue
p,p′-DDD	Para, para dichlorodiphyldichloroethane
p,p′-DDE	Para, para dichlorodiphyldichloroethylene
p,p′-DDT	Para, para dichlorodiphenyltrichlorethane
USEPA	United State Environmental Protection Agency

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