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Multi-residue study of pesticides in cocoa beans produced from Ghana using multivariate analysis

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ABSTRACT

Multi-residue levels of pesticides were determined in 44 distinct fermented dried cocoa beans samples collected from two cocoa beans storage warehouses located in Tema and Takoradi; cities in Ghana from November, 2010 to January, 2011. The main objective of this study was to evaluate, in the first place, the relationship between the levels of the pesticides in fermented dried cocoa beans, and then identify their mutual concentration dependence to identify their source. To achieve this, residue data obtained from gas chromatography mass spectrometry analysis of cocoa beans was subjected to multivariate analysis; specifically principal component analysis and cluster analysis. The extracting solvent was a pesticide grade acetonitrile, with two solid phase extraction clean-up cartridges; bond Elut C18 and Envi-carp/LC-NH_2 cartridges used for extract clean-up. The targeted compounds include but not limited to Beta-HCH, Alpha-Endosulfan, Endrin, p,p'-DDD, Dimethoate, Chlorpyrifos, Allethrin, Bifenthrin, Fenvalerate, Cyfluthrin, and Cypermethrin. Multivariate analysis of the residue data on fermented dried cocoa beans in R-mode and Q-mode grouped the detected pesticides into current use, previously used, run-off and drift from neighbouring crops pesticides applications. It also grouped sampled cocoa beans into four major clusters based on similarities in crop storage and agricultural farming practices. From the results, it was realized that future pesticides residues monitoring could be design to save cost, by selecting only marker pesticides from each identified groupings.

KEYWORDS

Pesticide;
Multi-residue;
Fermented Cocoa beans;
Gas chromatography;
Mass spectrometry;
Multivariate analysis.

INTRODUCTION

Cocoa beans originate from the pod of the tropical cocoa tree; *Theobroma cacao*. The technique of fermentation for cocoa beans, amidst other quality factors employed in the cocoa industry in Ghana, make cocoa beans produced from Ghana enjoy high premium on the World Commodities Markets[1]. This makes cocoa one of the biggest foreign exchange earners for Ghana; and it share in Ghana's GDP rose from 4.9% in 2000-2004 to 8.1% in 2005/2006[2]. However, one area of food safety that cost a fortune for Ghana's cocoa in 2006, when 60% of all consignment of cocoa beans exported to Japan were rejected is pesticide residue[3].

Pesticide is any substance or mixture of substances intended for preventing, destroying or controlling of any pest, including vectors of human or animal diseases, unwanted species of plants or animals causing harm[4]. However, when a crop is treated with a pesticide, a very small amount of the pesticide, or indeed what it changes to in the plant (its metabolites or degradative products), can remain in/on the crop until or after it is harvested. This is known as pesticide residue[5].

Contamination of cocoa beans with pesticide can occur via two ways. It can occur directly by treating the crop with pesticides before harvest, storage and distribution. It can also occur by the uptake from the soil of residual pesticides of the subsequent cocoa farming, from the atmosphere or drifting from neighbouring fields or from a storage space pretreated with pesticides[6].

Determination of pesticide residues in foodstuff is demanding with many analytical steps and much time being spent on reference samples and quality assurance work. Analytical consumable materials are expensive and many at times difficult to obtain, particularly for laboratories in certain developing countries. And for multi-residue methods like the one for fatty matrices such as cocoa beans, large quantities of consumables may be require for certain methods of choice[7]. It is therefore important to characterize pesticide residues trends for evaluation of the temporal variations of cocoa beans contamination. The usual practice of cocoa beans for residues assessment is the comparison of measured pesticide residues with threshold values recommended by international bodies or country's regulations, like the European Union, Japanese, etc[8,18-20]. In this wise, and considering the extensive export of Ghana's cocoa to Japan, the analytical method as developed by the Department of Food Safety, Ministry of Health, Labour and Welfare, Japan[9] was used in this study.

The present study was undertaken to evaluate, in the first place, the relationship between the levels of the pesticides in the medium cocoa beans, and then identify their mutual concentration dependence to identify their source. In situations like this, multivariate techniques such as Principal Component Analysis (PCA) and Cluster Analysis (CA) have been applied successfully in other earlier studies[10-14]. In conjunction with CA and PCA, this approach provides a means for ensuring source identification for a given pesticide distribution pattern in cocoa beans or any other medium[15-16]. Therefore to achieve the objective of the present study, residue levels of selected pesticides including but not limited to beta-HCH, alpha-endosulfan, endrin, p,p'-DDD, dimethoate, chlorpyrifos, allethrin, bifenthrin, fenvalerate, cyfluthrin, and cypermethrin were determined by gas chromatographic technique. The data was subjected to the multivariate analysis using simple and multiple correlations, together with apportionment analysis. It was envisaged that this approach would provide a basis to evolve correlation patterns of various pesticides in cocoa beans, which in turn would be useful for developing a cost effective control mechanism towards abatement of gross pesticide contamination in the selected area. The present study, the first of its kind in cocoa beans, would bring out pesticides source apportionment in residue detected in cocoa beans from Ghana, subsequently leading to the control of excessive agrochemicals by suitable processes.

MATERIALS AND METHODS

Sampling sites

Tema is locally nicknamed the "Harbour City" because of its status as a seaport. The port of Tema handles 80% of the nation's import and export cargo. It is a city on the Atlantic coast of Ghana, coordinated 5°40'N 0°0'W5.667°N 0°E, lying 25 kilometres east of the Ghanaian capital city of Accra, in the Greater

Accra region. Tema is one of Ghana's two deep seaports; Sekondi-Takoradi is the other. The Port of Takoradi is located 2 kilometres from centre of the city and coordinated on 4°53'5"N 1°44'26"W, in the Western Region of Ghana[17].

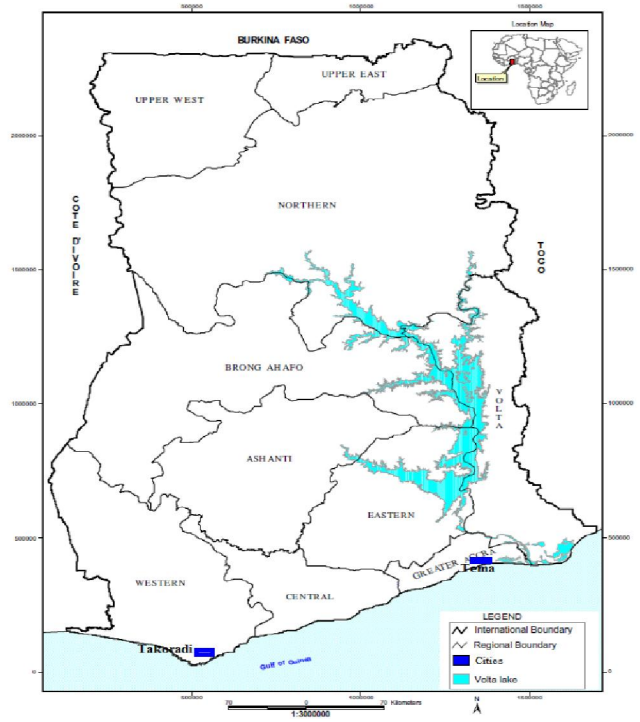

Figure 1 : Map of Ghana showing sampling locations (Tema and Takoradi)

Sampling plan

Fermented dried cocoa beans ready for export were stored in the warehouses. The beans were bagged in jute sacks each weighing about 63.5kg. Fermented dried cocoa beans ready for export were sampled at random from the two main cocoa warehouse stations located in Tema and Takoradi. At Tema, a total of twenty-four (24) distinct bagged samples each weighing about a kilogram of fermented dried cocoa beans, were sampled from November, 2010 to January, 2011. These were identified from seven (7) registered certified cocoa buying companies labeled A to G, and from the six cocoa growing regions of Ghana (Western, Eastern, Ashanti, Brong Ahafo, Central and Volta regions). The same cocoa buying companies and growing regions were identified at Takoradi station, with a total of twenty (20) distinct samples collected from November to December, 2010, and bagged in labeled zip lock plastic bags.

In all, a total of forty-four (44) distinct fermented dried cocoa beans samples, ready for export were sampled, labeled accordingly and were transported to the laboratory in five different batches.

Reagents and chemicals

Reagents used in the study comprised the following: Acetonitrile (Pesticide grade, BDH, England), Acetone (Pesticide grade, BDH, England), Acetone (Analytical grade, BDH, England), Ethyl Acetate (Pesticide grade, BDH, England), Toluene (Pesticide grade, BDH, England), Sodium sulfate (Pesticide grade, Aldrich-Chemie, Germany), Sodium chloride (Pesticide grade, Riedel-de Haen), dipotassium hydrogen phosphate (Analytical grade, BDH, England), Potassium dihydrogen phosphate (Analytical grade, BDH, England), Envi-carb/LC-NH_2 (500mg/500mg/6mL – Supelco), Strata C18-E (55um, 70A, 1000mg/6mL – Phenomenex) and distilled water.

The individual certified reference standards grouped into organochlorine pesticides: lindane, beta-HCH, delta-HCH, aldrin, heptachlor, gamma-chlordane, alpha-endosulfan, p,p'-DDE, dieldrin, endrin, beta-endosulfan, p,p'-DDT, p,p'-DDD, endosulfan sulfate, methoxychlor; organophosphorous pesticides: methamidophos, phorate, fonofos, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, malathion, fenitrothion, parathion, chlorfenvinphos, profenofos; and synthetic pyrethroids pesticides: allethrin, fenpropathrin, bifenthrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate and deltamethrin used for the identification and quantification were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Analytical methodology and instrumentation

Extraction and clean-up

Extraction and clean-up of cocoa beans samples were carried out according to procedures described by multi-residue method for agricultural chemicals by GC/MS from the Department of Food Safety, Ministry of Health, Labour and Welfare, Japan with slight modifications[9]. 20 mL of distilled water was added to 10.0 g of ground fine dried cocoa beans powder sample and allowed to stand for 15 minutes. 50 mL of acetonitrile was added and the sample homogenized using the ultra turax macerator. It was then centrifuged at 3000 rpm

and filtered into a 100 mL volumetric flask. An additional 20 mL of acetonitrile was added to the residue, and homogenized, centrifuged and filtered. Both filtrates were combined, and acetonitrile was added to make up a 100 mL solution. 20 mL aliquot of the extracted solution was then measured, and 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0) were added. It was then shook vigorously for 10 minutes on a horizontal shaker and allowed to stand for 10 minutes until the solution was clearly separated into layers. The aqueous layer was discarded.

An octadecylsilanized silica gel mini column (1000mg/6mL) was conditioned with 10 mL of acetonitrile. The acetonitrile layer from the above was then loaded onto the column, and the column eluted with 2 mL of acetonitrile afterwards. The entire volume of effluent was dry over anhydrous sodium sulfate, and filtered. The filtrate was concentrated to dryness at 40°C or lower and the residue re-dissolved in 2 mL of acetonitrile/toluene (3:1) mixture.

A second SPE, graphite carbon/aminopropylsilanized silica gel layered mini column (500mg/500mg/6mL) was conditioned with 10 mL of acetonitrile/toluene (3:1) mixture. The solution obtained from above extraction step was then loaded onto this column, and the column eluted with 20 mL of acetonitrile/toluene (3:1) mixture afterwards. The entire volume of effluent was then concentrated to 1 mL or less at 40°C or lower. 10 mL of acetone was added to the concentrated solution and further concentrated to 1 mL or less at 40°C or lower. A further 5 mL of acetone was added to the concentrated solution and then concentrated to dryness. The residue was re-dissolved in ethyl acetate to make a 1 mL solution, and was made ready for residue determination by GC-MS.

Instrumentation

This was as described in[18-20]. A Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with 1177 type injector, Saturn 2200 Mass Spectrometer (MS) as detector and a Varian 8400 autosampler were used for gas chromatography analysis. Sample extract of 2 µL aliquot was injected and the separation was performed on a fused silica gel capillary column (VF- 5ms, 30 m + 10 m column guard x 0.25 mm id., 0.25 um film thickness). The carrier gas was ultra pure helium at flow rate of 1.2 mL/min. The temperature of the injector operating in splitless mode was 270°C and the MS detector with an Ion trap mass analyzer was set to scan mass range between 40 m/z – 450 m/z at auto EI. The temperature of the manifold, ion trap and transferline were set at 80°C, 210°C and 260°C, respectively. The column oven temperature was programmed as follows; 70°C for 1 min, then at 30°C/min up to 240°C and finally at 5°C/min to 300°C held for 2.3 min. The total run time for a sample was 30 minutes. The residue levels of all detected pesticides were quantitatively determined by the external standard method using their peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times and spectra coincided with the reference standards were extrapolated on their corresponding calibration curves to obtain their respective concentrations.

Data analysis

Statistical analyses incorporated in the work include mean of samples, minimum and maximum values and corresponding standard deviation. Ranges were compiled from minimum and maximum values for levels detected in each individual organochlorine, organophosphorous and synthetic pyrethroids pesticide residues detected in the study. Residue data from fermented dried cocoa beans was subjected to Principal Component Analysis (PCA) to infer the hypothetical sources of pesticides. Factor Analysis (FA, the components of the PCA) was performed by Varimax rotation. Varimax rotation was employed because orthogonal rotation minimizes the number of variables with a high loading on each component and therefore facilitates the interpretation of PCA results. Cluster Analysis (CA) was applied to identify different groups, clustering the samples with similar pesticide residues contents. CA was formulated according to the Ward-algorithmic method, and the squared Euclidean distance was employed for measuring the distance between clusters of similar residue contents and also between certified cocoa buying companies. The mean of samples, maximum values and corresponding standard deviation were determined using XLSTAT 2011 software, whereas PCA and CA were performed by SPSS version 16 software for windows. All other calculations were per-

formed using Microsoft excel. However, only pesticides with percentage of detection more than 25% were subjected to the multivariate analysis.

RESULTS AND DISCUSSION

Cluster analysis (CA)

CA was performed on the cocoa beans residue data set. The residue data was normalized using Ward's method of linkage with squared Euclidean distance as a measure of similarity[21]. Cluster analysis used all the variance or information contained in the original residue data set. Ward's method was selected for pesticides/sampling site classification because it possesses a small space distortion effect, uses more information on cluster contents than other methods, and has been proved to be extremely powerful grouping mechanism[22]. All pesticides screened were coded as follows: first two letters of pesticide grouping by chemical nature, followed by the two letters from the pesticide's name. For example, Alpha-endosulfan, an organochlorine pesticide was coded as OC.AE; while Cypermethrin, a synthetic pyrethroids pesticide was coded as SP.CY and Dimethoate, an organophosphorous pesticide was coded as OP.DM. Cluster analysis in R-mode was performed on measured pesticides and two distinct groups or clusters were revealed. Cluster 1 contains OC.AE, SP.CY, OP.CH, OC.EN and OP.DM as Alpha-endosulfan, Cypermethrin, Chlorpyrifos, Endrin and Dimethoate, respectively. The pesticides Fenvalerate, Cyfluthrin, Aldrin, Bifenthrin, p,p'-DDD and Beta-HCH as SP.FE, SP.CF, OC.AL, SP.BI, OC.PD and OC.BH, respectively were grouped into cluster 2 (Figure 2). Cluster 1 consists of two organochlorine pesticides: Alpha-endosulfan and Endrin; two organophosphorous pesticides: Chlorpyrifos and Dimethoate, and one synthetic pyrethroids pesticide: Cypermethrin, a registered pesticide for cocoa production in Ghana[23]. With cluster 2, three organochlorines: Aldrin, Beta-HCH and p,p'DDD, a metabolite of the parent compound DDT; and three synthetic pyrethroids pesticides were grouped together with no organophosphorous pesticide. The synthetic pyrethroids pesticides in cluster 2 were Fenvalerate, Cyfluthrin and Bifenthrin; the latter two are well known pesticides among pesticides importers in Ghana[23].

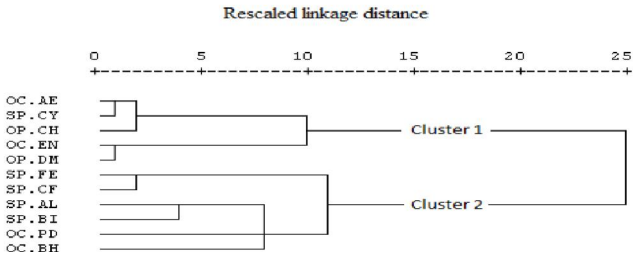

Figure 2 : A dendogram of measured pesticides residues in cocoa beans

All screened cocoa beans samples were coded as follows: first two letters of the city from where the cocoa beans were sampled followed by the letter representing the certified cocoa buying company and a number representing the count of cocoa beans samples from that particular buying company. For instance, the second cocoa beans sample from certified buying company labeled A sampled from Tema station was coded TE.A2, whiles the third cocoa beans sample from certified buying company labeled D, sampled from Takoradi station was coded TA.D3, and so on.

The Q-mode CA grouped all 44 sampled fermented dried cocoa beans into four statistically significant clusters (Figure 3). Group 1 consists of TE.A1, TA.A1, TA.A2, TA.B1, TA.B2, TA.B3, TE.C3, TA.C2, TA.C1, TA.D1, TE.F2, TE.F3, TA.F3, TE.G1, TE.G3, TE.G4, TE.G5, TE.G6 and TA.G1. Group 2 consist of only six of the samples; TE.A2, TE.D1, TE.E1, TA.E1, TA.F2 and TE.G2. Group 3 consist of TE.A3, TA.A3, TE.B1, TE.B2, TE.B3, TA.C3, TA.D2, TA.D3, TA.E3, TA.E2, TA.F1 and TA.G2. And Group 4 consists of TE.C1, TE.C2, TE.D2, TE.D3, TE.E2 and TE.F1 samples (Figure 3). All Group 1 samples were identified from six certified buying companies (A, B, C, D, F and G), and from all the six cocoa growing regions in Ghana (Western, Central, Ashanti, Eastern, Brong Ahafo and Volta Regions). However, Group 2 consists of samples from only three of the cocoa growing regions (Western, Ashanti and Brong Ahafo Regions), and from five of the certified cocoa buying companies (A, D, E, F and G). Group 3 consists of samples from all selected certified cocoa buying companies (A to G), but only from three cocoa growing regions of Ghana (Western, Ashanti and Brong Ahafo Regions). The fourth Group (Figure 3) also comprised of samples from three out of the six cocoa growing regions (Ashanti, Western and Central Regions) but from four certified cocoa buy-

ing companies (C, D, E and F).

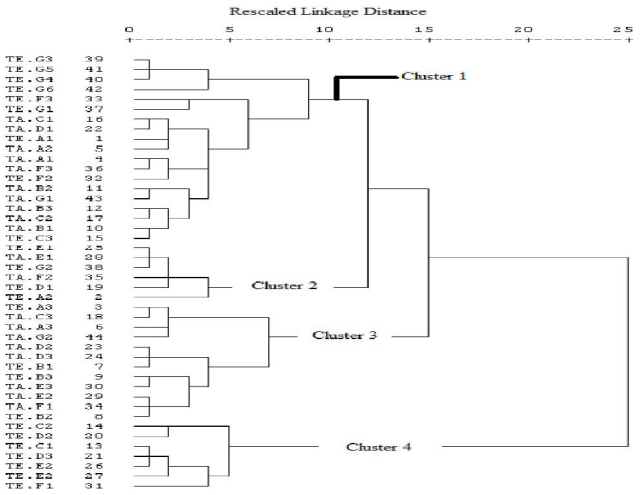

Figure 3: A dendogram of all screened fermented dried cocoa beans samples

Principal component analysis (PCA)

PCA is a powerful pattern recognition technique that attempts to explain the variance of a data set of inter-correlated variables with a smaller set of independent variables (principal component)[24]. PCA in R-mode was performed on the logarithmic form of pesticide residues data. Varimax rotation was used to maximize the sum of the variance of the factor coefficients.

TABLE 1 : Rotated component matrix of four factor model explaining 66.947% of the total variance for major detected pesticides

	PC 1	PC 2	PC 3	PC 4
OC.BH	***-0.634***	-0.002	-0.155	-0.100
OC.AE	**0.806**	0.156	-0.036	-0.040
OC.EN	0.210	**0.803**	0.077	0.102
OC.PD	-0.125	0.251	***-0.511***	0.158
OP.DM	0.081	**0.873**	-0.093	-0.035
OP.CH	**0.553**	0.305	-0.469	-0.015
SP.AL	-0.108	-0.174	0.152	**0.807**
SP.BI	-0.002	0.326	-0.195	**0.770**
SP.FE	***-0.607***	0.207	**0.498**	0.272
SP.CF	-0.067	0.172	**0.867**	0.097
SP.CY	**0.837**	0.209	-0.065	-0.231
Eigenvalue	2.509	1.840	1.586	1.429
Percentage of total Variance	22.811	16.728	14.417	12.991
Cummulative percentage of Variance	22.811	39.539	53.956	66.947

The results of the R-mode PCA are presented in (TABLE 1) with significant factor loadings in bold typed face.

Four principal components were obtained with Eigenvalues greater than one, explaining 66.947% of the total variance in pesticide residue data set (TABLE 1). The first principal component (PC1) was correlated with OC.AE, OP.CH and SP.CY. However, OC.BH and SP.FE with negative values were also significant but in reversed order to OC.AE, OP.CH and SP.CY (TABLE 1). This probably means as Alpha-endosulfan, Chlorpyrifos and Cypermethrin residues were on the increase, Beta-HCH and Fenvalerate decreased and vice versa. The second principal component (PC2) was correlated primarily with OC.EN and OP.DM, whereas the third principal component (PC3) was weighted on SP.FE and SP.CF with OC.PD in reversed trend. The fourth principal component (PC4) was correlated with SP.AL and SP.BI.

The factor scores from the R-mode PCA are given in TABLE 2. These classified the sampled cocoa beans from warehouses and buying companies according to the residue concentrations of the detected pesticides. For PC1, the pesticides Alpha-endosulfan, Chlorpyrifos and Cypermethrin are highly concentrated in samples TE.A2, TA.A2, TE.B1, TE.B3, TA.D2, TA.D3, TE.E1, TA.E1, TA.E2, TA.E3, TE.F3, TA.F1, TA.F2 and TE.G2 with factor scores of 1.083, 1.488, 0.747, 0.708, 0.893, 0.854, 1.270, 1.267, 0.888, 0.940, 1.392, 1.051, 0.714 and 1.987 respectively (TABLE 2).

The PC2 represents strong correlation for Endrin and Dimethoate for the following sampled beans in the order; TA.G2 > TA.C3 > TA.D3 > TA.E2 > TE.B2 > TE.D3 > TA.F1 > TE.E2 > TE.C2 > TA.A3 > TE.A3 (TABLE 2). PC3 also correlated strongly for the Synthetic Pyrethroids Pesticides Fenvalerate and Cyfluthrin for the following sampled beans in the order; TE.F1 > TE.A2 > TE.E3 > TE.C2 > TE.D3 > TE.E2 > TE.G2 > TA.E1 > TE.E1 > TE.C1 > TE.D2 > TA.G2. And finally PC4 shows strong correlation for the pesticides Allethrin and Bifenthrin (synthetic pyrethroids pesticides) for sampled beans TE.C2, TE.D2, TE.F3, TA.F1 and TE.G1 with factor scores of 1.781, 3.259, 2.727, 1.495 and 1.993, respectively.

Pesticides source identification with PCA

The R-mode PCA performed on the pesticide resi-

TABLE 2 : Varimax rotation factor scores for the four factor model for the analysed fermented dried cocoa beans

	PC1	PC2	PC3	PC4
TE.A1	-0.115	0.117	-0.215	-0.271
TE.A2	**1.083**	0.057	**1.841**	-0.600
TE.A3	0.380	**0.748**	-0.953	0.637
TA.A1	-0.269	0.586	-0.966	-0.860
TA.A2	**1.488**	-0.379	-0.551	0.136
TA.A3	0.413	**0.816**	0.555	-0.930
TE.B1	**0.747**	0.256	0.547	-0.694
TE.B2	0.612	**1.244**	-0.853	0.556
TE.B3	**0.708**	-0.083	0.299	0.467
TA.B1	-0.346	0.408	-0.646	-0.136
TA.B2	-1.231	0.119	-1.182	0.880
TA.B3	-0.478	-0.437	-0.554	-0.959
TE.C1	-1.762	0.544	**0.847**	-0.332
TE.C2	-1.605	**0.841**	**1.632**	**1.781**
TE.C3	0.014	-0.178	-1.024	0.381
TA.C2	-0.407	-0.220	-0.594	-0.569
TA.C3	0.129	**1.842**	0.076	-0.020
TE.D1	0.468	-1.967	-0.261	-0.453
TE.E2	-0.776	**0.886**	**1.708**	-0.286
TA.E1	**1.267**	-1.661	**1.229**	-0.354
TA.E2	**0.888**	**1.463**	-0.305	0.922
TA.E3	**0.940**	-0.007	-0.218	0.384
TE.F1	-0.976	-1.428	**2.141**	-0.044
TE.F2	-0.572	-0.650	-1.603	-0.569
TE.F3	**1.392**	-0.685	-0.247	**2.727**
TA.F1	**1.051**	**0.939**	-0.902	**1.495**
TA.F2	**0.714**	-0.948	0.740	-0.825
TA.F3	-0.478	0.129	-1.242	-0.686
TE.G1	0.120	-1.216	-1.350	**1.993**
TE.G2	**1.987**	-1.257	**1.292**	-0.286
TE.G3	-0.892	-1.251	-0.455	-0.762
TE.G4	-1.126	-1.392	-0.375	-0.835
TE.G5	-0.974	-1.189	-0.363	-0.810
TE.G6	-1.782	-0.702	-1.639	-0.503
TA.G1	-0.752	-0.331	-0.242	0.231
TA.G2	0.503	**2.159**	**0.688**	-1.165

dues data set revealed four latent factors. These factors were responsible for the data structure, explaining 66.947% of the total variance of the data set and possibly from current use, previous use, run-off or drift.

PC1 has the highest Eigenvalue of 2.509 (TABLE 1). An Eigenvalue gives a measure of the significance for the factor, and the highest Eigenvalue is the most significant[25]. The pesticides Alpha-endosulfan, Chlorpyrifos and Cypermethrin, and inversely Beta-HCH and Fenvalerate registered factor loadings greater than 0.5 and constituted absolute loading values. PC1 may be interpreted as representing influences from current use of these pesticides in cocoa production in Ghana mainly from farming and crop storage practices. PC2, which is next in significance with an Eigenvalue of 1.840, is strongly correlated with Endrin and Dimethoate. Dimethoate has been identified as mixed formulation with Cypermethrin, an approved pesticide for use on cocoa production in Ghana. Examples of these formulations include Cymethoate Super EC, Cypadem 43.6% EC and Cyperdicot EC[23]. Cocoa farmers may have used these formulations, and as a matter of fact had contributed greatly to PC2 percentage of total variance of 16.728 (Table 1). The third principal component, PC3, has an Eigenvalue of 1.586 and is third in significance. This pole has the pesticides Fenvalerate, Cyfluthrin and inversely p,p'-DDD as its members. PC3 may be attributed to previous use of those pesticides. This could be true since Fenvalerate, PC3 member, was also in PC1 in an inverse correlation order. And if PC1 was identified with current use of Alpha-endosulfan, Chlorpyrifos, and Cypermethrin, and inversely with Beta-HCH and Fenvalerate, then in PC3 it could possibly be from previous used pesticide. Again p,p'-DDD is a metabolite of the parent compound DDT, and since DDT was not among PC1 current used pesticides, it confirms PC3 as previously used pesticide. PC4 with the least Eigenvalue of 1.429 contributed 12.991% of the total variance in residue data. It contains two insecticides; Allethrin and Bifenthrin which may be attributed to pesticides drift considering their least significance to the PCA and their approval among pesticides used in Ghana[23].

R-mode CA was performed on residue data set to ascertain similarities among the various pesticides. The R-mode CA reveals two distinct pesticides groupings (Figure 2). The results suggest that the pesticides which fall in a particular group share similar characteristics. The cluster analysis has, therefore, provided a useful classification of the pesticides in the study, which could be used as pesticides markers to design an optimal future monitoring work with lower cost. On the basis of

the result from this work, the number of monitoring pesticides could be reduced and chosen only from Groups 1 and 2.

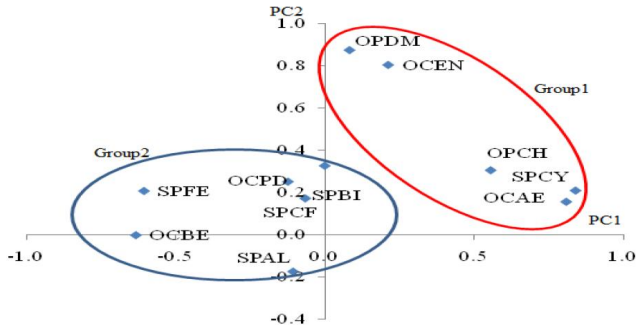

Figure 4 : A plot of principal component PC1 versus principal component PC2 for factors scores in R-mode PCA

Figure 4 shows the factor scores of pesticides examined on the bi-dimensional plane defined by PC1 and PC2, and clearly distinguishes 12 pesticides into two groups. Group one contains OP.MD, OC.EN, OP.CH, OC.AE and SP.CY, while group two consists of SP.FE, OC.PD, SP.BI, SP.CF, OC.BE and SP.AL. Comparing these groupings to R-mode CA indicates very strong agreement in the groups (Figure 2 and Figure 4). This confirms the fact that the pesticides in each group posses' similar characteristics. A closer look at Figure 4 shows that group one may be divided into two groups. The first group containing OP.DM and OC.EN, and the second comprises OP.CH, SP.CY and OC.AE. This groups the pesticides into current use, previously used and drift sources as demonstrated by the PCA.

Similarities among sampled cocoa beans using PCA and CA

Q-mode CA was performed on residues data set to ascertain similarities among the various sampled beans. The Q-mode CA defines groups (clusters) of sampled beans of a particular region and company in terms of the examined pesticides. The Q-mode CA reveals four distinct sampled groupings (Figure 3). The results suggest that the sampled beans which fall in a particular group share similar characteristics with respect to the analysed pesticides. The cluster analysis has, therefore, provided a useful classification of the cocoa beans samples in the study area, which could be used to design an optimal future monitoring sampling plan with lower cost. On the basis of the result from this work, the number of monitoring sampled cocoa beans could be reduced and chosen only from Groups 1, 2, 3 and 4. The similarities and differences within the sampled beans from the warehouses was also investigated by the factor scores of the R-mode PCA. Figure 5 shows the factor scores of sampling points on the bi-dimensional plane defined by PC1 and PC2, and clearly distinguishes the 44 sampled cocoa beans into four groups.

Group 1 consists of TA.A1, TA.B1, TE.A1, TA.F3, TA.C2, TE.C3, TA.G1, TA.B3, TE.F2, TE.D2, TE.G5, TE.G3, TE.F1, TE.G4 and TE.G1. Group 2 consists of TA.A2, TE.F3, TA.F2, TE.E1, TA.E1, TE.G2 and TE.D1. TA.G2, TA.C3, TA.D3, TA.E2, TE.B2, TA.A3, TA.C1, TA.F1, TE.A3, TA.D2, TE.B1, TA.D1, TE.A2 TE.B3 and TA.E3 were together in Group 3 (Figure 5). The PCA Group 4 comprised TE.D3, TE.C2, TE.E3, TE.C1, TE.E2, TA.B2 and TE.G6. Comparing these with the CA, it also clustered sampled cocoa beans into four major groupings (Figure 3). Between group 1 of PCA and CA; TA.A1, TA.B1, TE.A1, TA.F3, TA.C2, TE.C3, TA.G1, TA.B3, TE.F2, TE.G5, TE.G3, TE.G4 and TE.G1 were common.

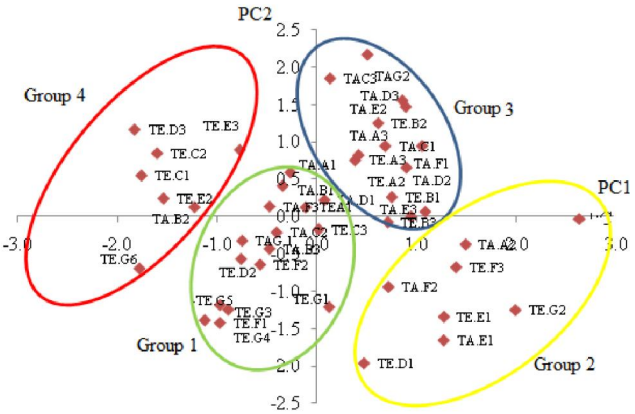

Figure 5 : A plot of principal component PC1 versus principal component PC2 for R-mode

TE.E1, TA.E1, TE.G2, TA.F2 and TE.D1 were common to group 2 PCA and CA. Common samples in group 3 between PCA and CA consists of TA.G2, TA.C3, TA.D3, TA.E2, TE.B2, TA.A3, TA.F1, TE.A3, TA.D2, TE.B1 and TE.B3. And in group 4, TE.C2, TE.C1, TE.D3, TE.E2 and TE.E3 were common between PCA and CA grouping. The good agreement between PCA and CA for sampled cocoa beans confirms the fact that the forty-four sampled cocoa beans can be grouped into four main groups with similar characteristics. Thus, samples in the same group come from

the same sources. It could also possibly be that those samples were treated with the same farming and crop storage practices.

CONCLUSION

Multi-residues levels of pesticides in cocoa beans produced in Ghana as determined by Gas chromatography coupled with Mass Spectrometry shows significantly varying results:

The present work demonstrates the usefulness of the statistical multivariate methods towards classifying the pesticides as groups in terms of their independent behaviour and identifying their probable sources. Multivariate analysis on the pesticide residue data of fermented dried cocoa beans in R-mode and Q-mode grouped the detected pesticides into four latent factors; current use, previously used, run-off and drift from neighbouring crops pesticides applications. It also grouped sampled cocoa beans into four major clusters based on similarities in crop storage and agricultural farming practices. It is therefore anticipated, that any feasible futuristic solution to pesticides contamination problem in fermented dried cocoa beans from the four classified sampled groups should only be based on selected pesticides in the two groups as revealed by the cluster analysis (CA) and confirmed by the principal component analysis (PCA).

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