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Original Article

Statistical correlations between mineral element composition, product information and retail price of powdered cocoa beverages in Nigeria

T.A. Shittu [a,*], B.A. Badmus [b]

[a] Department of Food Science and Technology, University of Agriculture, Alabata Road, Abeokuta, Nigeria
[b] Department of Home and Rural Economics, College of Agriculture, PMB 1025, Jalingo, Taraba State, Nigeria

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ABSTRACT

A study of the mineral element composition, labelled product information and retail prices of 30 Nigerian made powdered cocoa beverages (PCBs) was carried out. The objective was to determine statistical correlations between retail price, mineral element composition and the practice of PCB manufacturers towards providing product information as an index of good manufacturing practice (GMP). The products were generally found to be rich sources of Ca and P. Fe, Cu and Cr were found in trace amounts. Most PCB sample had Pb content above the maximum permissible level of 1.0 μg/g, The calcium content correlated significantly with phosphorus content ($r = 0.919$, $P < 0.01$) and GMP score ($r = 0.639$, $P < 0.01$). Based on products' mineral element composition, the use of some multivariate pattern recognition techniques successfully clustered the PCB brands into two main groups. The first group consisted mainly of unregistered samples with GMP scores ≤ 8 (maximum score = 12) while the second group consisted mainly of registered products with GMP scores ≥ 8. Based on the mineral element composition, the extreme positions of Pb and P in the clustering tree indicated that they are contaminant and nutritional supplements, respectively. Finally, the retail prices, Ca and P contents were shown to be reliable indices of GMP in PCBs.

1. Introduction

During the International Union of Food Science and Technology (IUFoST) Governing Council Meeting, Santiago, Chile, in the year 2000, serious food quality and safety concerns were raised about the developing world (Winarno, 2000). Food safety was also identified as the most important factor in International food trade, while good manufacturing practices (GMP) were identified as one of the key invisible factors that determine safety of food commodities. GMP is defined as the minimal sanitary requirements that must be met by a food plant (Troller, 2005). Apart from the general sanitary requirement published in Codex standards (CAC, 1991), local regulatory authorities have the prerogative of re-defining non-complying products with a minimum of worldwide requirement based on some local factors. Assessment of nutritional quality and safety of food consumed by the public is an important tool for the development of nutritional and health policies in a nation.

Heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) are generally regarded as environmental contaminants, and their presence in foods can have some toxic effects in human. High levels of Pb in foods may result to food poisoning in humans either in acute or chronic exposure. Accumulation of lead produces damaging effects in the hemato-poetical, hematic, renal, gastrointestinal systems (Correia et al., 2000; Baht and Moy, 1997). Zinc plays a role as an integral part of a number of metalloenzymes and as a catalyst for regulating the activity of over 300 specific zinc-depended enzymes (McCall et al., 2000). Chromium is also an important element, and controls certain physiological process in human body with respect to blood insulin and lipid profile. Excessive consumption of chromium is rare since it is poorly absorbed (Mertz, 1993). The role that copper plays in anemia is related to the mobilization of the tissue iron, the formation of the mitochondrial heme, and the reduction of erythrocyte half-life (Conrad and Umbreit, 2000). Both copper deficiency and excess result in anemia (Ramirez-Cardenas et al., 2005). Copper has no pro-oxidant activity at a considerably higher intake (Rock et al., 2000). Diets low in copper are suggested as an explanation for much of the epidemiology and patho-physiology of ischemic heart disease (Klevay, 2000). However, the presence of Cu and Zn in foods has both nutritional and toxic effects in humans.

All of these elements become toxic when concentrated in food (Celik and Oehlenschlager, 2005). Entry of deleterious chemicals into cocoa product is thus a concern to the regulatory authorities.

* Corresponding author. Tel.: +234 803 538 8060; fax: +234 39 243031/233031.
E-mail addresses: staofeek0904@yahoo.com (T.A. Shittu), rabayoor2004@yahoo.com (B.A. Badmus).

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Hence, determination of the actual concentration of these elements in food is essential. In a recent study by Rankin et al. (2005), it was established that globally most Pb contamination of cocoa and chocolate products occurs during shipping and/or processing of the cocoa beans.

Nigeria is the largest cocoa-growing nation in the world, next to Cote d'Ivoire and Ghana, with about 366,000 Mt of cocoa beans produced in the year 2004 (FAO, 2005). As at year 2000, only six out of the seventeen cocoa manufacturing plants in the country were operating at less than 40% combined installed capacity (GAIN, 2000). The current situation is still unknown. Cocoa powder is the major primary product made from cocoa beans in Nigeria, and the greatest portion of the powder is consumed locally as either powdered cocoa beverage (PCBs) or used as raw material for food flavoring in some other manufacturing processes. Due to the relatively simple and low cost technology required in making PCBs, it has attracted the attention of cottage processors as a feasible food-processing venture. This has also caused the nation to experience an influx of many cottage-manufactured brands of PCBs into the food market. Consequently, the relevant regulatory agencies like NAFDAC face the additional challenge of constantly carrying out surveillance to control spurious manufacturing activities. Despite this effort, independent studies are needed to establish indices that could be used by consumers to authenticate the status of any of these products in the food market.

Assessment of GMP through regular visit of manufacturing sites by the agents of regulatory authorities to ensure compliance may not be totally realistic in some situations, especially in developing economies due to a number of reasons. Firstly, scattering of manufacturing plants over undisclosed places constitutes a major impediment to regulatory activities. In addition, some cottage food manufacturers that operate with minimum GMP requirement find it difficult to register with relevant regulatory authorities due to some economic and bureaucratic factors. Therefore, a more realistic approach to GMP assessment is product-based. This includes determination of product identity, and analysis of the compositional and safety qualities through the retail marketing outlets. General requirement for the declaration of product identity has been provided in the CODEX General Standard for the Labelling of Prepackaged Foods (CAC, 1991). This requires detailed declaration of product composition and nutritional information, manufacturing and expiry date and vivid address of manufacturing site on the retail packs.

This paper sets out to examine to what extent PCB manufacturers in Nigeria have complied with product-based GMP. It also attempts to correlate the products' heavy trace metal composition, declared product identity and retail prices of the products using statistical techniques.

2. Methods and materials

2.1. Beverage sample collection

A total of 30 different brands of locally produced PCB were randomly picked from retail outlets in four major markets in Ibadan, Abeokuta and Lagos which are the major commercial centers in the south west of Nigeria. During sample collection, the products' retail prices were recorded. Other information was also recorded, such as the number of ingredients listed, as well as information on nutritional composition, sample weight, presence and completeness of manufacturer's address. The information on registration status or product's approval by the relevant regulatory body such as National Agency for Food Drug Administration and Control (NAFDAC) provided on the products' packages were also noted. The GMP scores were made based on the presence or absence of this information, except the number of ingredients declared, which is not a mandatory aspect of the product's manufacture but rather the individual company's product initiative and/or innovation. Complete information was

Table 1
Assessment of product-based GMP scores and retail prices of the powdered cocoa beverage samples.

Sample	Registration status	Presence of address	Completeness of address	Address authentication	Nutritional information	Manufacturing/ expiry date	GMP score	Retail price (N/g)
1. B/CAD242	2	2	2	2	2	2	12	0.598
2. CBC-231	2	2	2	2	2	2	12	0.586
3. MON-253	2	2	2	2	2	2	12	0.906
4. BC-646	2	2	2	2	-	-	8	0.571
5. NV-288	2	2	2	2	-	-	8	0.498
6. ST-311	1	2	2	2	1	1	9	0.08
7. BT-299	1	2	1	1	1	1	7	0.075
8. BM-156	1	1	1	1	1	1	6	0.072
9. SC-112	2	2	2	2	-	-	8	0.379
10. RS-191	1	2	2	2	1	1	9	0.344
11. BD-737	1	2	-	-	-	-	3	0.177
12. BN-730	1	2	1	1	1	1	7	0.079
13. MT2-954	1	2	2	1	1	1	8	0.237
14. VC-145	–	–	–	–	–	–	0	0.082
15. ML-264	1	2	1	1	1	1	7	0.12
16. NV-196	1	2	2	1	1	1	8	0.229
17. BO-223	1	2	2	1	1	1	8	0.085
18. GT-101	1	2	2	1	1	1	8	0.234
19. BG-551	–	–	–	–	–	–	0	0.08
20. BT-272	1	2	1	1	1	1	7	0.097
21. BU-205	1	2	1	1	1	1	7	0.072
22. GP-214	1	2	1	1	1	1	7	0.077
23. LT-179	1	2	1	1	1	1	7	0.083
24. EB-160	1	2	2	1	1	1	8	0.237
25. GC-843	1	2	1	1	1	1	7	0.079
26. MT-662	1	2	2	1	1	1	8	0.089
27. UA-123	1	2	2	2	1	1	9	0.19
28. MV-28	1	2	1	1	1	1	7	0.245
29. DC-134	1	2	1	1	1	1	7	0.353
30. BV-188	1	2	1	1	1	1	7	0.177

given a maximum of two marks; partial inclusion was given one mark; and absence was given a zero mark (Table 1).

2.2. Reagents

The concentrated acids $HClO_4$, HNO_3, and H_2SO_4 (BDH, Laboratory Supplies, UK) used were of analytical grade. Stock standard solution (1000 ppm) of calcium (Ca), iron (Fe), lead (Pb), copper (Cu) and chromium (Cr) were used in preparing their calibration curve after some serial dilutions. Standard phosphate solution was prepared by dissolving dry anhydrous KH_2PO_4 in 1000 ml de-ionized water and stored in dark brown Pyrex glass bottle. Molybdate reagent was also prepared by dissolving 20 g molybdate [$(NH_4)_6MO_7O_{24}{\cdot}4H_2O$] in 200 ml hot de-ionized water. Twenty-gram meta-vanadate was also dissolved separately in 120 ml of hot de-ionized water solution. The vanado-molybdate reagent was prepared by mixing the molybdate and vanadate solution and further diluted to 1000 ml.

2.3. Sample treatment

A 125 ml Erlenmeyer flask was washed with $KClO_4$ acid solution and distilled. Samples (0.5 g) were mixed with 4 ml of perchloric acid, 25 ml concentrated HNO_3 and 2 ml concentrated H_2SO_4 under a fume hood. The content was heated gently on a hot plate under a fume hood until no trace of carbon was found in the digested sample. The digested sample was further heated for about 30 s, cooled and filtered using Whatman no 42 filter paper. The solutions were stored in refrigerator for 2–6 h until ready for use.

2.4. Chemical analysis

The digested samples were flamed to determine Ca, Fe, Pb, Cu, P and Cr contents in a digital atomic absorption spectrophotometer (VGP 210, Buck Scientific, Connecticut, USA). Phosphorus content was determined by measuring the % transmittance at 4000 nm.

2.5. Quality control

All samples were analyzed in triplicate. In order to reliably establish the GMP of the manufacturers, we ensured that the PCB samples that had difference batch references on their packages were sampled at three different retailers. This was impossible for those that had no batch reference. However, they were carefully obtained from different retailers who stocked it at different times.

2.6. Statistical analysis of data

Experimental data were analyzed using one-way analysis of variance (ANOVA) and Duncan's mean separation. The data was also explored in recognizing some pattern for classification and discrimination purposes using multivariate techniques (principal component (PCA) and cluster analysis (CA)). SPSS 10.0 and S-Plus 2000 statistical packages were used in data analysis.

3. Results and discussion

Table 2 shows the list of ingredients combined in formulating the PCB brands studied. The basic ingredients commonly declared on the labels are cocoa powder, sugar and milk powder. The labels also indicated that use of flavorants is common. Some manufactures also endeavored to further indicate the kind of flavorants used. The number of ingredients declared ranged from 3 to 10. One notable feature of the declarations made is that some technical inaccuracy abounds. For example, some manufacturers declared inclusion of sugar while glucose was separately mentioned. The commonest sugar used in this kind of product is crystalline sucrose. Therefore, when other types of sugars have been used, it would have been proper to specify these types of sugar parenthetically if they must be listed. Some declarations, which appeared to be fraudulent, were also found. This violates the CODEX requirement for cocoa powders and dry mixtures of cocoa powder and sugars (CAC, 2001), which specifically prohibited the inclusion of flavoring agents that reproduce the flavor of cocoa or milk. For example, two of the manufacturers declared the inclusion of chocolate flavor. It is suspected that such declaration may either be an error or merely establishing that the cocoa powder used is of poor quality thereby necessitating augmentation of the cocoa flavor with some cocoa flavor concentrate. It is also interesting to note that one manufacturer did not declare sugar as part of ingredients despite the significant amount of sugar that is often added to such products. None of the labels carried the word 'sweetener' as part of the ingredients declared.

Product labelling is also a fraction of indices of GMP. Accurate product labelling sells a product by giving the customer sufficient confidence about the authenticity and quality of the product. The results of GMP assessment are shown in Table 1. Our findings showed that over 73% of the products were unregistered; only one of the products had no address printed on the package. However, about 43% of the manufacturers' declared addresses were incomplete. The incompleteness lies in the fact that they lack pertinent items like street number; when the addresses were carefully traced it was discovered that about 64% of the products carried addresses that were not authentic. Some 70% of the products did not carry nutritional labelling. About 7% did not declare ingredient composition. The maximum score obtainable from our assessment scale for GMP, as explained above, is 12. The assessment scores for the retailed PCB brands ranged from 0 to 12.

Table 2
List of ingredients declared on the packages of the Nigerian-made powdered cocoa beverages.

Product brand	ingredients
B-CAD242	Cocoa powder, skimmed milk powder, sugar, glucose, malt extract, milk protein, emulsifier, vitamins, minerals
CBC-231	Cocoa, sugar, skimmed milk powder, vegetable fat, permitted flavors, vitamins, minerals
MON-253	Cocoa powder, skimmed milk powder, malt extract, glucose syrup, vegetable oil, vitamins, minerals
SC-112	Low fat cocoa, sodium chloride, vitamins, minerals, flavor
RS-191	Low fat cocoa, sugar, milk, sodium chloride, edible starch, flavor
NV-288	Cocoa, sugar, milk, vanilla flavor
VC-145	Cocoa, sugar, vitamin, milk, flavor
EB-160	Cocoa, vitamin, milk flavor, sugar
ST-311	Low cocoa fat, milk, sugar, salt, vitamin, flavor
LT-179	Cocoa, sugar, milk, flavor, salt
BT-272	Cocoa, sugar, milk, flavor, salt
BM-156	Cocoa, sugar, milk, vanilla flavor
MV-28	Cocoa, sugar, vanilla flavor, minerals
GT-101	Cocoa, sugar, milk, flavor, vitamin, salt
MT-662	Cocoa, sugar, milk, flavor, minerals
BO-223	Cocoa, sugar, milk, vanilla flavor, vitamin A
DC-134	Cocoa, sugar, milk, flavor, minerals
GC-843	Cocoa, sugar, milk, chocolate flavor, vitamins
BU-205	Cocoa, sugar, skimmed milk powder, egg, lecithin, vitamin
BD-737	Cocoa, sugar, milk, vanilla flavor, minerals
BV-188	Cocoa, sugar, milk, minerals
BG-551	Cocoa, sugar, milk, vanilla flavor, minerals
UA-123	Cocoa, sugar, skimmed milk, chocolate flavor, salt
GP-214	Cocoa, sugar, milk, flavor
ML-254	Cocoa, sugar, milk, flavor
BT-272	Cocoa, sugar, milk, flavor, salt
BN-730	Cocoa, sugar, flavor, other

There were two brands that scored 0 points, while two brands scored the maximum of 12 points.

The retail price of a manufactured product partly determines the potential sales volume of the product. It is often expected to reflect the manufacturing cost per unit of the product. In ensuring GMP, a substantial amount of fund is set aside to enhance process and product quality. Thus, it may be hypothesized that market prices of products is a reflection of the quality of the product. Table 1 shows the retail price of the PCB samples studied. The retail price of the PCB samples studied varied significantly, ranging from 0.07 to 0.906 N/g. Sample 3 had the highest while samples 8 and 21 had the least. Out of all the variables, the correlation between retail price and GMP score was the highest ($r = 0.864$, $p < 0.01$).

The list of PCB brands and mineral element composition are shown in Table 3. Calcium and phosphorus were the most abundant element in the cocoa powders. Their composition ranged from 14 to 574 and 0 to 671.0 mg/100 g, respectively. Copper, chromium, iron and lead were found in trace amount with concentrations ranging from 0.3 to 3.2, 0 to 0.32, 9.5 to 65.2 and 0.09 to 0.38 mg/100 g, respectively. The lead content of most of the PCB brands is well above the maximum permissible level (MPL) of 1.0 ng/g (0.1 mg/100 g) recommended by the Cocoa Producer's Alliance (COPAL, 2004). The mean Pb content for product having GMP score $\geq$8 was 0.222 mg/100 g compared to 0.275 mg/100 g for those with GMP score $\leq$6. This may also imply that PCBs manufactured from companies observing GMP code are likely to have reduced risk of lead contamination in their products.

At present, the rate of consumption of PCBs by Nigerians is not known. The knowledge of this could have assisted us to estimate the level of risk to which the consumers of each product are exposed. In spite of this, consumers should be apprehensive of the chronic exposure to Pb accumulation, which is the major pathway of toxicity. It has been reported that Cu is required for iron utilization, and as a co-factor for enzymes involved in glucose metabolism and synthesis of hemoglobin, connective tissue and phospholipids (Ramirez-Cardenas et al., 2005; Celik and Oehlenschlager, 2005). The amount of each element we found in the PCB samples are very similar to those reported by Daini et al. (2003) in Nigeria and Pedro et al. (2006) in Brazil except that the phosphorus content of the beverages studied here were unusually higher. One distinct observation is that those beverage samples that contained high amount of calcium were similarly high in phosphorus. Cr content was higher in some samples than those reported by previous authors, while in most samples the Cr content was so insignificant that it could not be determined by the analytical method used here. The correlation between Ca and P contents is 0.919 ($p < 0.01$). Also, the samples that were scored zero for GMP were characteristically having no detectable level of phosphorus. The correlation between P content and GMP score is positive ($r = 0.719$, $p < 0.01$). However, retail price correlated mostly with GMP score ($r = 0.783$, $p < 0.01$) among other variables studied.

Table 3
Mineral element composition (mg/100 g) of powdered cocoa beverage samples.

Sample	Ca	Fe	Pb	Cu	Cr	P
1. B/CAD242	116.8	26.2	0.36	1.59	ND	218.2
2. CBC-231	152.9	31.6	0.28	0.39	ND	247.8
3. MON-253	572.3	27.0	0.12	1.38	0.32	671.0
4. BC-646	63.8	28.1	0.19	1.79	0.24	199.4
5. NV-288	92.3	18.3	0.25	0.79	0.28	163.5
6. ST-311	62.3	22.2	0.21	0.99	ND	ND
7. BT-299	29.9	18.5	0.14	0.59	ND	ND
8. BM-156	22.3	65.2	0.29	0.59	ND	ND
9. SC-112	21.7	24.6	0.10	0.78	0.14	127.9
10. RS-191	21.1	23.9	0.19	2.58	ND	39.8
11. BD-737	19.7	27.0	0.22	1.39	ND	39.8
12. BN-730	19.5	18.7	0.29	1.37	ND	ND
13. MT2-954	19.2	18.2	0.28	1.91	0.19	19.8
14. VC-145	19.4	18.6	0.27	0.6	ND	ND
15. ML-264	19.2	15.8	0.21	1.58	ND	89.0
16. NV-196	18.2	19.4	0.09	1.2	ND	20.0
17. BO-223	18.8	24.6	0.08	0.79	ND	ND
18. GT-101	18.0	25.6	0.31	1.99	0.17	40.0
19. BG-551	17.3	20.9	0.32	0.79	ND	ND
20. BT-272	17.6	18.6	0.15	0.59	0.12	163.5
21. BU-205	16.8	20.3	0.17	1.8	ND	ND
22. GP-214	16.4	20.3	0.33	0.98	ND	ND
23. LT-179	16.5	15.3	0.31	0.99	0.05	ND
24. EB-160	16.3	16.4	0.22	1.18	0.22	88.8
25. GC-843	15.7	19.3	0.24	1.39	ND	ND
26. MT-662	15.7	25.8	0.16	0.39	ND	10.0
27. UA-123	15.5	15.5	0.38	0.4	ND	39.8
28. MV-28	15.3	20.9	0.19	0.99	ND	58.8
29. DC-134	14.9	15.7	0.24	0.4	ND	109.1
30. BV-188	14.9	9.5	0.22	0.79	0.06	ND

ND, means not determined.

Naturally, cocoa and cocoa powders are low in Ca and P content, contrary to what was found in the PCB samples. The Ca and P content must have been intentionally supplemented by the respective manufacturers; this could have partly been responsible for the relatively higher retail prices of those products containing high level of Ca and P. Cr, Cu and Fe may be conveniently classified as microelements. Apart from soil and genetic factor, other possible sources of variation in these micronutrients are in the use of fertilizer, agrochemical and processing equipment.

To better understand the variability and similarity among the PCBs samples using analytical data, we decided to use some multivariate pattern recognition techniques. Principal component analysis (PCA) is a multivariate technique that has been used to identify variables useful in identifying the similarity and differences among the PCB samples (Shittu and Lawal, 2007). The PCA was carried out using both the correlation and covariance matrices, respectively. The first principal component (PC1) generated from the covariance matrix was dominated by P contributing about 99.9% of the total variation while the second component was dominated by Ca. and it contributed about 0.1% of the total variation (Fig. 1). The remaining elements except Cu were located in the third component which virtually contributed insignificantly to the total variation among the PCBs mineral element composition

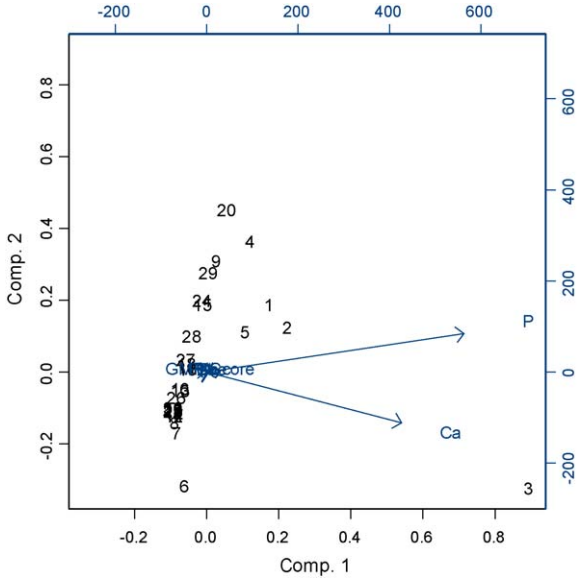

Fig. 1. Principal component loading plot of mineral element composition, GMP score and retail prices of cocoa beverages using covariance matrix. The graph shows the relationship between variables and sample in space.

Table 4
The factor loading of the first three principal components using covariance and correlation matrices of the variables.

Variable	Principal component[a]					
	Covariance matrix			Correlation matrix		
	1	2	3	1	2	3
Ca	0.604	−0.797	–	0.467	−0.158	–
Fe	–	–	1.000	–	−0.710	−0.234
Pb	–	–	–	−0.125	−0.239	−0.761
Cu	–	–	–	0.113	0.575	−0.580
Cr	–	–	–	0.274	0.261	–
P	0.797	0.604	–	0.500	−0.111	–
Price	–	–	–	0.484	–	−0.109
GMP Score	–	–	−0.999	0.352	–	–
Variance (%)	95.93	3.75	0.29	45.24	14.08	12.67

[a] Loading values greater than 0.1 are shown.

(Table 4). It is clear that Ca and P are the elements that contributed mostly to the variability among the thirty PCB samples (Fig. 1). Notably, samples 1, 2, 3, 4, 5 and 20 are distinctly different from the remaining in terms of Ca, P contents and retail prices. However, from the correlation matrix (Table 4), it could be concluded that virtually all the variables had significant loading on the first two PCs. The first PC contributed about 47% of the variations while the second PC explained only about 14% of variations. The correlation between Ca, P and retail price was very high as shown in Fig. 2.

In order to clearly show the partition between the PCB samples, the Ca, P and retail price were selected as classification variables and subsequently were used in clustering the PCB brands. The PCB samples were clustered into two major groups labelled A and B. Group B consisted of only one brand (Sample 3) and is only 75% similar to the others in Group A, which consists of the remaining 29 brands of PCB. Group A membership is also divided into groups C and D. The samples in each group are about 97.5% similar to each other. The outlying nature of sample 3 may be due to its unusually very high phosphorus content (671.0 mg/100 g).

The registration status of each product brand was indicated by marking each as either registered (R) or unregistered (U). Samples 1, 2, 3, 4 and 5 were separated from the other in the clustering tree (Fig. 3). Despite the fact that sample 20 was unregistered it had P content similar to those of registered status. However, it is not as similar in terms of retail price (Table 4). Equally, Sample 9 which had a registered status was clustered with the unregistered samples due its low P content. When hierarchical cluster of mineral elements was generated as shown in Fig. 4, it confirmed the close relationship observed between Ca and P earlier.

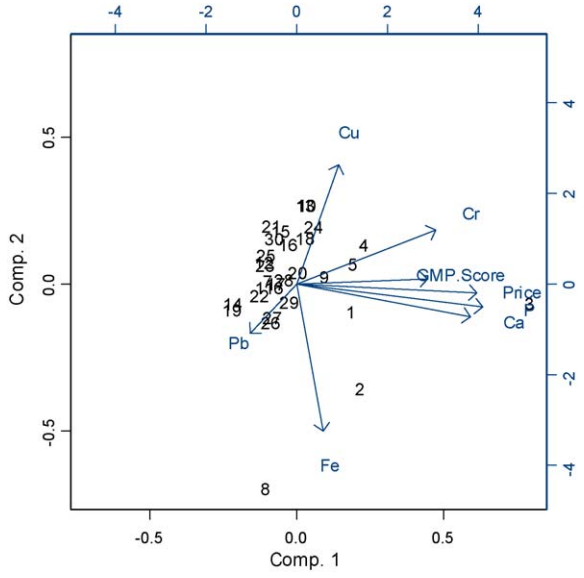

Fig. 2. Principal component loading plot of mineral element composition, GMP score and retail prices of cocoa beverages using correlation matrix. The graph shows the relationship between variables and sample in space.

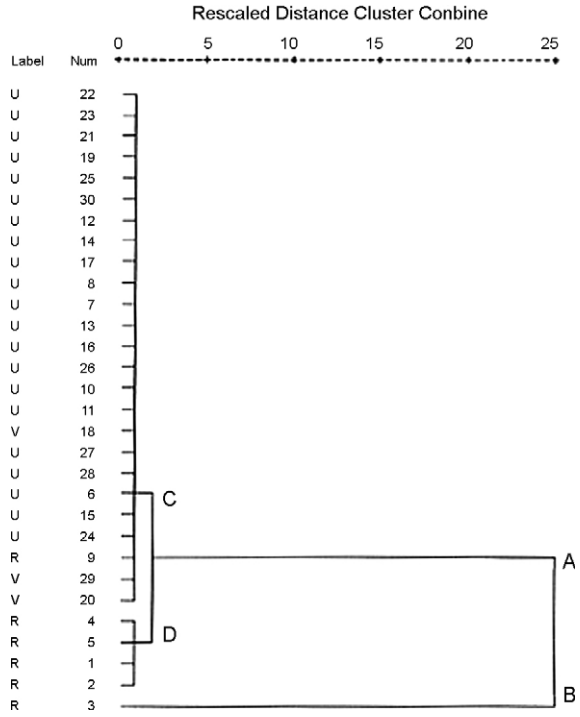

Fig. 3. Classification based on Ca, P and price with dendrogram using average linkage (between groups). U: unregistered sample; R: registered sample.

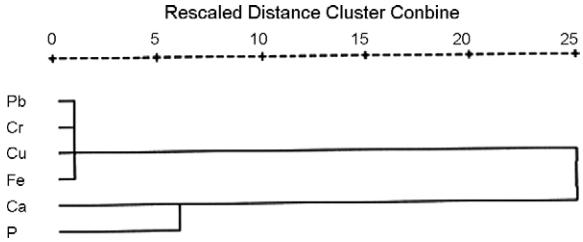

Fig. 4. Hierarchical cluster of elements found in the powdered cocoa beverage samples using average linkage (between groups).

4. Conclusions

This study has shown that the majority of the powdered cocoa beverage brands retailed in Nigeria lacked proper product identity. Similarly, most of them were not registered with the designated regulatory authority. Despite these results, the products were found to be generally rich in Ca and P, and low in Cu, Fe and Cr. The Ca content correlated significantly with P content. There exist a highly significant correlation between P content and retail price per gram of the products. Finally, there was a significant positive correlation between Ca and P contents, and the GMP score. Therefore, Ca and P content could be used as indices of GMPs in powdered cocoa beverage manufacture. In addition, consumers should be apprehensive of the quality of PCBs with retail prices too low for the product. Pb only appeared to be an extraneous element in the entire beverage samples since its level in the products did not correlate with any of the variable studied.

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