Relationship between Viable Bacterial Counts and Physicochemical Properties of Cocoa Powders and Powdered Cocoa Beverages purchased in Nigerian Supermarkets.

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ABSTRACT: Cocoa powders (CPs) and powdered cocoa beverages (PCBs) are largely consumed as health and vitality drinks in Nigeria. In view of the recent reports that these food products supported bacterial growth, this study determined whether there could be definite relationship between viable bacterial counts and physicochemical properties of five brands of CPs and PCBs purchased from supermarkets in Ibadan and Lagos in South West, Nigeria. The pH and titratable acidity of the products were measured by using pH meter and colorimetric acidity titration respectively. The moisture contents were determined by drying method at 105ºC in oven and ash by mineralization at 550ºC using furnace. No definite type of relationship was established between viable bacterial counts and pH, titratable acidity (lactic acid, acetic acid), moisture and ash contents vis-à-vis powdery and granular natures of the cocoa food products. Only the two PCBs tightly packed in the polythene sachets had acceptable mean viable bacterial counts, which did not exceed 5.0 x 10^3 cfu/ml specified for cocoa powders in food industries. Also, only two of the five brands had acceptable moisture contents, which did not exceed the national specification of 3.10% for cocoa powders. Furthermore, the ash contents of all the brands exceeded the acceptable national specification of 2.60%. Significant variations ($P<0.05$) were obtained in the mean pH, titratable acidity, viable bacterial counts, moisture and ash contents within and between products containing only cocoa powders and those containing additives. In conclusion, the results from this study showed that the physicochemical properties of cocoa powders and powdered cocoa beverages could not be used to predict the viable bacterial counts of these food products.

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1. INTRODUCTION

Cocoa powders (CPs) and powdered cocoa beverages (PCBs) are largely consumed in Nigeria as health and vitality drinks, refreshment food drinks and dietary supplements to enrich the foods of women after child delivery, sick people and sports men and women (Dada *et al.,* 1982; Ebuehi & Disu, 2000; Jayeola & Oluwadun, 2010).

The recent increase in the consumption of cocoa food products has been ascribed to the on going awareness campaign that has shown cocoa to prevent age related health problems, fatigue, hypertension, diabetes, breast cancer, cardiovascular and mental diseases (Keen *et al.*, 2005; Hollenberg, 2006; Grassi *et al.*, 2006; Olubamiwa, 2007).

The manufacturing process of cocoa powder begins with the cleaning, roasting, breaking and winnowing of fermented dried cocoa beans to produce cocoa seeds or nibs. The germ is separated from the nib and then milled to form cacao-mass or chocolate liquor, which is subjected to alkalization, fat pressing, breaking, grinding and sifting to form cocoa powder, which is the main ingredient for producing cocoa-based beverages.

However, cocoa food products generally can be contaminated by the food handlers with a wide variety of microorganisms during manufacturing and packaging processes. Recent reports have shown that cocoa powders and powdered cocoa beverages are good culture media for bacteria and fungi despite their low moisture content and powdery or granular nature (Ogunledun 2007; Adesina *et al.*, 2010; Jayeola & Oluwadun, 2010).

According to Hildrum *et al*., (1984), the analysis of food quality has been characterized in the past by single univariate measurements of single parameters, which may have several drawbacks. In microbiological analysis for instance, the principal drawback to standard plating methods for determining the number and type of microorganisms in a food product is the time required for pre-enrichment and incubation of the product and plates respectively. Therefore, there is need for the development of rapid and multivariate techniques for rapid assessment of microbiological quality of food products. Such innovation in food analysis will require more knowledge of how the physical and chemical factors of the food together affect the viable counts of microbes in it.

Hence, this study was carried out to give a scientific report on how the pH, titratable acidity, moisture and ash contents, packaging and physical states of cocoa powders and powdered cocoa beverages purchased in some supermarkets in Nigeria relate to the viable bacterial counts of the food products.

2. MATERIALS AND METHODS

2.1 Cocoa powders and powdered cocoa beverage samples

Different brands of cocoa powders and powdered cocoa beverages were purchased from the supermarkets in Ibadan, and Lagos in South West, Nigeria. The trade and manufacturers' names, ingredients and packaging methods of the cocoa products were obtained from the labels on the products and recorded. Laboratory size samples suitable for physicochemical and microbiological analyses were obtained according to the method described by Pomeranz & Meloan (1996).

2.2 pH Measurement

The pH of each dispersed cocoa powder suspension of 2% strength was determined at room temperature (29.7ºC) using electrodes of a pH meter (Hanna Instruments) placed directly into each suspension. The pH meter with accuracy of 0.1 was first standardized using buffer solutions of pH 4 and 9. The determination was performed in triplicate to find the mean pH of each sample.

2.3 Determination of Titratable acidity (TA)

The cocoa powder and cocoa-based beverages were assayed for titratable acidity (lactic acid and acetic acid) according to the method of Friedrich (2001) using colorimetric acidity titration as follows. Equal parts of deionized distilled water (ddH_2O) were added to the solid samples and macerated in blender at 100 rpm for 2 minutes before centrifuging for 5 minutes at 2500 rpm at room temperature. To 10ml of the supernatant sample solution in a clean Erlenmeyer flask were added 5 drops of 1% phenolphthalein indicator solution and a magnetic stir bar before stirring on a magnetic stir plate. Then 0.1N NaOH was carefully titrated against the sample solution to the endpoint of pH 8.2 until a faint but definite pink colour, which was stable for 5 to 10 seconds was obtained. The titratable acidity was calculated using the equation:

$$TA\ (g/100ml) = \frac{(V)(N)(meq.wt.)(100)}{(1000)(v_s)}$$

Where V is volume (ml) of NaOH solution used for titration; N is normality of NaOH solution; meq.wt is milliequivalent weights of acids: lactic acid = 90, acetic acid = 60; v_s is sample volume = 10ml. The analysis was performed in triplicate to find the mean titratable acidity of each sample.

2.4 Compositional analysis

The moisture contents of the cocoa powders and powdered cocoa beverages were determined by the oven drying method at 105ºC (AOAC, 1990). The ash contents of the cocoa products were determined after mineralization at 550ºC according to AOAC (1990).

2.5 Enumeration of viable bacteria

Viable bacterial count analyses were performed on samples of the cocoa products following the procedures of the International Commission for Specification for Food (ICMSF, 1978). A tenfold serial dilutions up to 10^{-6} for each sample were prepared in 0.1% peptone water and subsequently plated onto standard Plate Count Agar (PCA). The PCA plates were incubated at 37 ºC for 48h. The colony forming units (cfu) were counted on plates having between 30 and 300 colonies using Quebec colony counter. The enumeration of viable bacterial count was carried out in duplicate on each sample and the isolated bacteria were identified using Gram staining and catalase methods described by Cheesebrough (1985).

2.6 Statistical analysis

Results were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 15.0. The significant differences between means were determined at P<0.05.

3.0 RESULTS

3.1 Ingredient and packaging of cocoa products

Table 1 shows the trade names, ingredients manufacturing countries, and packaging of the five cocoa products. Four of the products were produced in Nigeria while one in Ghana. Two of the products, CR and BG, contained natural cocoa powder as the only ingredient while the remaining three (SC, CB and NM) contained additives such as skimmed milk powder, glucose, vanillin, vitamins or minerals. Two of the products (CB and NM) were enclosed in sachets made of thick polythene containers while two (CR and BG) were packed in plastic containers. The remaining brand, SC, was packed in a tin container.

3.2 Viable bacterial counts and physical factors of cocoa products

Table 2 shows that three of the cocoa products (CR, SC and BG) were in powdered form while the remaining two (CB and NM) were in granules. The mean viable bacterial count 1.2×10^4 cfu/ml of CR was significantly higher than the mean viable bacterial count of each of the remaining four cocoa products (P<0.05). There was a significant difference in the mean viable bacterial counts between CR and BG, which were both powdered cocoa products packed in plastic containers (P<0.05). Similarly, a significant difference was obtained in the mean viable bacterial counts between CB and NM, which were both granular powdered cocoa beverages packed in polythene sachets (P<0.05). The Gram staining and catalase test revealed that the bacteria were all Gram positive spore forming bacilli. Some of the bacteria were catalase positive while others were catalase negative.

3.3 Mositure and Ash contents of cocoa products

The moisture and ash contents in relation to viable bacterial counts of the cocoa products are presented in Table 3. The mean moisture content of the cocoa powders (CR, SC and BG) ranged between 2.00 – 6.50%, compared to 1.00 – 5.00% for the powdered cocoa beverages (CB and NM). A significant (P<0.05) variation in moisture content of samples was observed with CR powder having significantly highest moisture content of 6.50% and CB beverage having the significantly lowest moisture content of 1.00%. The mean ash contents of the cocoa powders ranged between 6.3 – 8.7% compared to 4.5- 5.8% for powdered cocoa beverages. A significant (P<0.05) variation in ash content of samples was also observed with SC powder having significantly highest ash content of 8.70% and NM beverage having the significantly lowest ash content of 4.50%. The significantly lowest (P<0.05) viable bacterial count of 4.0×10^3 was obtained in CB cocoa-based beverage.

3.4 pH and Titratable acidity of cocoa products

Table 4 presents the pH, titratable acidity (lactic acid and acetic acid) and viable bacterial counts of the cocoa products. Results revealed that the mean pH 7.60 of each of the CB and NM, which were all cocoa-based beverages was significantly higher than the mean pH values 6.00, 6.60 and 5.60 of the other products CR, SC and BG respectively which were cocoa powders (P<0.05). The two cocoa-based beverages also had the lowest titratable lactic acid (0.004%) and acetic acid (0.002%) when compared to the results obtained for the three cocoa powders. The viable bacterial counts of the cocoa powders were significantly higher than those of powdered cocoa beverages (P<0.05).

Table 1. Commercial cocoa powders and powdered cocoa beverages investigated.

Trade Name	Manufacturer	Ingredient	Packaging
CRIN Natural Powder (CR)	Cocoa Research Institute of Nigeria	Natural cocoa powder	Plastic
Spectra Cocoa Mix powder (SC)	Spectra Nig. Limited	Defatted cocoa powder, skimmed milk powder and vanillin	Tin
Brown Gold Natural Cocoa powder (BG)	Hords Limited, Ghana	Natural cocoa powder	Plastic
Cadbury Bournvita (CB)	Cadbury Nigeria Plc	Malt extract, sugar, glucose, Cocoa powder, skimmed milk powder, milk protein, Emulsifier (E472), vitamins and minerals	Sachet
Nestle Milo (NM)	Nestle Nigeria Plc	Malt, sugar, skimmed milk powder, glucose syrup, cocoa powder, vegetable oil, essential vitamins and minerals	Sachet

Table 2. Viable bacterial counts and physical factors of cocoa products.

Cocoa products	Packaging	Physical state	Bacterial count cfu/ml**
CR powder	Plastic	Powder	1.2×10^{4a}
SC powder	Tin	Powder	8.0×10^{3b}
BG powder	Plastic	Powder	6.0×10^{3c}
CB beverage	Sachet	Granule	4.0×10^{3d}
NM beverage	Sachet	Granule	5.0×10^{3e}

** Means of duplicate samples. Means having different superscripts within the column are significantly different at P<0.05.

Table 3. Viable bacterial counts, moisture and ash contents of cocoa products.

Cocoa products	Moisture in %*	Ash in %*	Bacterial count cfu/ml**
CR powder	6.50 ± 0.04^{a}	7.50 ± 0.06^{g}	1.2×10^{4a}
SC powder	4.00 ± 0.02^{c}	8.70 ± 0.05^{f}	8.0×10^{3b}
BG powder	2.00 ± 0.01^{d}	6.30 ± 0.02^{h}	6.0×10^{3c}
CB beverage	1.00 ± 0.01^{e}	5.80 ± 0.04^{i}	4.0×10^{3d}
NM beverage	5.00 ± 0.03^{b}	4.50 ± 0.01^{j}	5.0×10^{3e}

* Means of triplicate samples ± SD; ** Means of duplicate samples. Means having different superscripts within the column are significantly different at P<0.05.

Table 4. Viable bacterial counts and chemical factors of cocoa products.

Cocoa products	pH*	Titratable Acidity in % * Lactic acid	Acetic acid	Bacterial count cfu/ml **
CR powder	6.00 ±0.04[c]	0.043[c]	0.029[c]	1.2×10^{4a}
SC powder	6.60 ± 0.01[b]	0.029[b]	0.019[b]	8.0×10^{3b}
BG powder	5.60 ± 0.03[d]	0.058[d]	0.038[d]	6.0×10^{3c}
CB beverage	7.60 ± 0.08[a]	0.004[a]	0.002[a]	4.0×10^{3d}
NM beverage	7.60 ± 0.10[a]	0.004[a]	0.002[a]	5.0×10^{3e}

* Means of triplicate samples ± SD, ** Means of duplicate samples. Means having different superscripts within the column are significantly different at P<0.05.

4.0 DISCUSSIONS

Five brands of cocoa food products were purchased without any bias from the supermarkets in Ibadan and Lagos cities in South West Nigeria to investigate whether there could be any clear scientific relationship between their physicochemical factors and viable bacterial counts with the view of finding presumptive physical and chemical methods of predicting microbiological quality of these cocoa drinks. The investigated physicochemical factors of the products include packaging materials, physical state, pH, titratable acidity (lactic acid and acetic acid), moisture and ash contents while their microbiological qualities were determined using viable bacterial counts (Tables 1 to 4).

The significant differences obtained in the physicochemical parameters of the cocoa products is not surprising since they differ in ingredient formulation (Table 1). The CRIN natural Powder (CR) and the Brown Gold Natural Cocoa powder (BG) contained no other ingredient than natural cocoa while the Spectra Cocoa Mix powder (SC), Cadbury Bournvita (CB) and Nestle Milo (NM) comprised additives ranging from vanillin to glucose, milk powder, vegetable oil, essential vitamins and minerals. All these additional ingredients in the products are expected to change at least the chemical properties such as pH, titratable acidity, moisture and ash contents of the products.

Also, the differences in the physical states of the products and their packaging materials depicted in table 2 could be responsible for the significant differences in viable bacterial counts of the products. The CB and NM were powdered cocoa beverages in granular state tightly packed in thick polythene sachets. Each of them had a mean viable bacterial count, which did not exceed 5.0×10^3 cfu/ml specification for cocoa powder to be used as ingredient for the food industries (Katenburg and Muijnck, 1993). On the other hand the cocoa powders enclosed in large plastic and tin containers had viable bacterial counts exceeding the specification for cocoa powder. In fact, CR exhibited significantly higher bacterial count of 1.2×10^4 cfu/ml when compared to the results of other products. The large plastic and tin containers with wide mouths will permit entry of more air, moisture and bacteria from the air into the products during their packaging and subsequent opening during laboratory analysis. All these could be responsible for their high viable bacterial counts.

Only CB and BG in table 3, with moisture contents of 1.00% and 2.00% respectively met the national standard being enforced by NAFDAC (1995), which stipulates that the moisture contents of cocoa food products should not exceed 3.10%. Also, all the five brands of the products exceeded the 2.6% of ash content prescribed by NAFDAC (1995) for cocoa powders and beverages. In relating the microbiological quality of the products to their physicochemical properties, a direct relationship was observed between the mean moisture content and the mean bacterial count in CB, which had the lowest values of 1.00% and 4.0×10^3 cfu/ml for the two parameters respectively vis-à-vis other brands. Similar direct relationship was obtained between the mean ash contents and bacterial counts of CR and SC both of which contained very high ash contents of 7.50% and 8.70% respectively and very high mean viable bacterial counts of 1.2×10^4 and 8.0×10^3 respectively.

Ash is the inorganic residue from the incineration of organic matter. The amount and composition of ash in a food product depend on the nature of the food ignited and on the different proportions of the various minerals that compose the ash and on the method of ashing (Pomeranz & Meloan, 1996). The various minerals that compose ash in foods include calcium, phosphorus, iron and magnesium, which have been reported to be present in relatively high concentrations in cocoa powders and powdered cocoa-based beverages sold in markets in the South-West Nigeria (Ogunledun, 2007; Jayeola & Oluwadun, 2010).

In Table 4, significant variations were obtained in pH and titratable acidity values of the products ($P<0.05$). Also, an inverse relationship was observed between the mean values of pH and titratable acidity (lactic acid and acetic acid) on one hand and the viable bacterial count on the other hand. The significantly lower pH values ranging from 5.60 to 6.00 and higher values of lactic acid and acetic acid ranging from 0.043% to 0.058% and from 0.029% to 0.038% respectively obtained in CR, SC and BG could be due to activities of lactic acid and acetic acid bacteria. These bacteria were isolated as Gram positive spore forming bacilli on the standard plate count agar (PCA) used in the viable bacterial count of the products. The findings are not surprising because during cocoa fermentation, yeasts, lactic acid bacteria and acetic acid bacteria develop in succession (Thompson *et al.* 2001). The lactic acid bacteria convert glucose in the cocoa into lactic acid during fermentation (Vuyst, 2000).

Dried cocoa food product can be described as a complex ecosystem consisting of itself as the abiotic environment and the microorganisms that live in it as the biotic factors. When microbes are placed in environments below or above neutrality their ability to proliferate depends upon their ability to bring the environmental pH to a more optimum value or range. When placed in acidic environment, the cells must either keep H^+ ions from entering or expel them as rapidly as they enter. Such key cellular compounds as DNA and ATP require neutrality (Brock *et al.,* 1984). Also, when most microbes grow in acidic media their metabolic activities result in the media or substrates becoming less acidic, while those that grow in high pH environments tend to effect a lowering of pH.

In conclusion, the findings of this study have shown that there are no precise boundaries for minimum and maximum moisture and pH of cocoa powders and powdered cocoa beverages since the actual values seem to be dependent upon other bacterial growth parameters such as ash content. Unless a very high degree of sanitation is employed in the cocoa powder and beverage manufacturing process, the low moisture content and pH imparted by drying and acid respectively will not be sufficient to ensure low viable bacterial counts in these food categories. However, more future analyses of the substances in cocoa food products that are responsible for the adverse pH against viable bacterial counts will contribute to ensuring food safety. In view of the high hygroscopic nature of cocoa, dry products should be tightly enclosed in thick polythene sachets like those used for Cadbury Bournvita (CB) and Nestle Milo (NM) to reduce absorption of moisture from air by the cocoa food products in order to extend their shelf life.

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