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ASSESSMENT OF MICROBIOLOGICAL AND CHEMICAL QUALITIES OF SELECTED COCOA, TEA AND COFFEE BRANDS IN NIGERIAN MARKETS

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ABSTRACT
Background: Cocoa, Tea, and Coffee products are consumed worldwide; they are rich in nutrient and, thus, prone to microbial contaminations that could cause food infections and intoxication. Objective: the objectives of the paper are: To evaluate the microbial and chemical qualities of some popular brands of cocoa, coffee and tea products in Nigerian market and benchmark it with standard specifications, to determine the products safety for human consumption and proffer solutions on ways to prevent possible food borne hazards associated with these products. Materials and Methods: This study examined the microbiological and chemical qualities of some brands of these products at the consumer level using standard analytical methods. Results: Five (50%) of the cocoa products had coliform counts (cfu/g) significantly ($p<0.05$) higher than acceptable limits and range from $2.6\times10^{1}\pm0.01$ to $4.6\times10^{1}\pm0.01$. The mean total aerobic plate count and fungal counts (cfu/g) cocoa, coffee, and tea were not significantly ($p<0.05$) different from standard specifications. The microbial isolates include species of *Bacillus* (59.2%), *Staphylococcus*(12.0%), *Enterobacter*(1.6%), *Aspergillus*(20.0%), *Penicillium*(14.4%) and *Saccharomyces* (12.0%). Moisture contents higher than 6% and 3% standard specifications in tea and cocoa products was detected in 7(70%) of tea and 2(20%) of cocoa products respectively. The samples are rich in phenol contents (mg/ml) and contain varying concentrations of manganese, calcium, iron, and copper. Free Radical Scavenging (DPPH) activity of 6.2±0.01 to 16.3±0.02µg/ml was detected in the samples. Conclusion: Some cocoa products contain unacceptable levels of coliforms, the high moisture contents above 3 and 6% standard specifications in some cocoa and tea products could encourage the proliferation of mycotoxigenic moulds and pathogenic bacteria to hazardous levels. The use of good raw materials, compliance to good manufacturing practices (GMP) and apt storage are advocated.
Keywords: Chemical qualities, Cocoa, Coffee, Microbial qualities, Mycotoxigenic moulds, Tea

ÉVALUATION DES QUALITÉS MICROBIOLOGIQUES ET CHIMIQUES DE CACAO, THÉ ET CAFÉ RÉPUTÉES DANS LES MARCHÉS NIGÉRIANS

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RÉSUMÉ
Contexte: Cacao, thé, café et produits sont consommés dans le monde et ils sont riches en nutriments et, par conséquent, sujettes aux contaminations microbiennes qui pourraient causer des infections et intoxications alimentaires. Objectif : les objectifs de l'étude sont : d'évaluer les qualités microbiologiques et chimiques de certaines marques populaires de cacao, café et thé produits en marché nigérian et comparaison avec les spécifications standard, pour déterminer l'innocuité des produits pour la consommation humaine et de proposer des solutions sur les moyens de prévenir les risques d'origine alimentaire associées à ces produits. Matériels et méthodes : Cette étude a examiné les qualités microbiologiques et chimiques de certaines marques de ces produits au niveau de la consommation à l'aide de méthodes analytiques.
Résultats: cinq (50 %) de la produits de cacao avait des coliformes fécaux (ufc/g) de manière significative ($p < 0,05$) plus élevée que les limites acceptables et vont de 2,6×101±0,01 à 4,6×101±0.01. La moyenne d'une numération totale sur plaque et le nombre de cellules fongiques (CFU/g) de cacao, café, thé et n'ont pas été significativement ($p < 0,05$) de différentes spécifications standard. Les isolats microbiens comprennent des espèces de Bacillus(59,2 %), Staphylococcus(12,0 %), Enterobacter(1,6 %), Aspergillus (20,0 %), Penicillium(14,4 %) et Saccharomyces(12,0 %). Contenu d'humidité plus élevé que 6 % et 3 % dans les spécifications standard de plateau et de produits de cacao a été détectée dans 7(70 %) de plateau et 2(20 %) de produits de cacao, respectivement. Les échantillons sont riches en matières de phénol (mg/ml) et contiennent différentes concentrations de manganèse, calcium, fer, et cuivre. De radicaux libres (DPPH) activité de 6,2 ± 0,01 à 16,3±0,02µg/ml a été détecté dans les échantillons.

Conclusion: Certains produits de cacao contiennent des niveaux inacceptables de coliformes, le contenu d'humidité élevée au-dessus de 3 et 6 % des spécifications standard dans certains produits de cacao et de thé pourrait encourager la prolifération des moisissures et bactéries pathogènes mycotoxigenic à des niveaux dangereux. L'utilisation de bonnes matières premières, de la conformité aux bonnes pratiques de fabrication (BPF) et de stockage sont préconisées. apt
Mots-clés: qualités chimiques, cacao, café, qualités microbiennes, moules, thé Mycotoxigenic

INTRODUCTION Cocoa, Tea, and Coffee have received much attention due to their significant roles in the human diet, the beverage/confectionery industries and high polyphenols contents. Products of cocoa, tea and coffee present varied microbial contamination levels and chemical contents and possess different levels of antioxidant potentials (1, 2, 3). To reduce contamination of coffee, tea and cocoa products, factors such as use of quality raw materials, correct storage conditions, application of hazard analysis, critical control points (HACCP) and other quality systems in good manufacturing practices (GMP), hygienic processing environment, training and education of food handlers should be well implemented. Cocoa, coffee, and tea contribute significantly to the economy of Nigeria and some African countries; they are a major non-oil foreign exchange earner for the countries: Cote d'Ivoire, Ghana, Cameroon, Uganda, Togo, Sierra Leone, Ethiopia, and Kenya and contribute substantially to the rural economy (4, 5, 6).There have been recorded outbreaks of food infections and intoxication, as a result of consumption of tea, coffee and cocoa products due to poor manufacturing practices or poor storage facilities (7, 8).The high level of unemployment consequent on poor economy/recession has lead to the emergence of large numbers of small scale home made products, packaged under substandard conditions and brought into the market without quality control/assessments to ascertain their health benefits and implications. Cocoa and coffee beans and tea can be contaminated with toxigenic fungal species and deleterious fungal toxins especially ochratoxin A and aflatoxins (7, 9, 10, 11, 12, 13, 14). Toxic elements of modern day environmental pollution fluoride, lead, and aluminum have also been found in tea (15, 17). Knowledge of nutritional, microbiological, chemical and biochemical compositions of food is important to health, well-being, and safety of the consumers (18) and to the manufacturers in understanding the importance of various nutritional contents so that a number of essential nutrients may be maintained or improved during and after processing. Beverages (coffee, tea, and cocoa) are nutritionally rich to support the proliferation of microbial spp (19) and contain polyphenols and minerals that have diverse beneficial biochemical, antioxidant and antimicrobial effects (20, 21, 22), This study is therefore set to assess the microbiological and chemical qualities of some cocoa, coffee and tea products in Nigerian markets.

METHODS

Sample collection

One hundred and twenty-five (125) samples made up of five each of ten different brands of cocoa and tea products and 5 different brands of coffee products were purchased from supermarkets in Ota (Ogun state) and Lagos (Lagos State) Nigeria. Ota in Ogun state is located at 6° 41′ 00″ N 3° 41′ 00″ E while Lagos is situated at 6° 27′ 14.65″ N 3° 23′ 40.81″ E. All the samples were collected within the months of February to July 2016 and all samples purchased are within expiry date. The samples were transported to the laboratory for analyses. The microbiological and phytochemicals analyses were carried out at the Microbiology and Biochemistry laboratories of Covenant University while the proximate and mineral analyses were assayed at the Research Laboratory of Bells University of Science and Technology, Ota, Ogun State. Prior to analyses, the manufacture/expiry dates, batch number and manufacturers address were documented.

Sample analysis

Microbiological analysis

Standard methods for microbiological analysis (23, 24)was adopted for Total Aerobic Plate Count (TAPC), Total Coliform Count (TCC), Total Fungal count (TFC), *Staphylococcus aureus* count (SAC), and isolation of some organisms of concerns such as Salmonellae, Shigella, Cronobacter, and moulds. Cultural characteristics and Biochemical tests and employing the API kits Biomerieux® sa, were used in the identification of the bacterial isolates. Fungal isolates were identified on the basis of their Macroscopic and Microscopic characteristic (25).

Chemical analysis

The method of the Association of Official Analytical Chemists (26) was adopted for determination of proximate compositions (moisture, ash, crude fibre, protein, fat, and carbohydrate). The mineral contents (elements) of the samples: calcium (Ca), magnesium (Mg), iron

(Fe) and copper (Cu) were determined using the atomic absorption spectrophotometer (G105 UV-VIS, Thermo Fisher Scientific, GeneSys, Madison, USA) as described in the methods of the Association of Official Analytical Chemists (26). The $AlCl_3$ method as described by Oranusi *et al* (27) was used for the determination of the total flavonoid content of the sample extracts. Total phenolics were determined using Folin-Clocalteus method following the description of Oranusi *et al* (27). The antioxidants scavenging activity of the samples on the stable free radical DPPH was measured by the method as described by Brand-Williams *et al,* (28).

Statistics

Results for microbial count parameters were presented as a mean and standard deviation, chemical compositions and microbial isolates were presented as percentages of occurrence. One way analysis of variance was employed to compare mean microbial loads and correlation analysis and test of significance for the proximate, mineral and microbial compositions for the three products (p=0.05).

RESULTS

Microbiological evaluation

The mean microbial counts (total aerobic plate count, *S. aureus* count, fungal count and coliform count) for cocoa, tea and coffee products are presented in Table1. It shows that all the samples have significantly ($p < 0.05$) low TAPC of the order 10^1 to 10^2. The cocoa and tea products were however relatively more contaminated (not significant at p=0.05) when compared to coffee samples, counts ranging from 1.7×10^2 - 2.9×10^2 cfu/g were obtained from cocoa products. Tea products had counts ranging from 1.7×10^2 - 4.5×10^2 cfu/g, while coffee products gave counts that ranged from 2.2×10^1 - 4.5×10^1 cfu/g. Mean *S. aureus* count of the samples reveals that most of the samples had significantly ($p < 0.05$) low counts of the order 10^1 to 10^2. The cocoa and tea products, however, had more counts when compared to coffee samples. Counts ranging from <10 - 7.6×10^1 cfu/g were obtained from cocoa products. Tea products gave counts of < 10 - 4.7×10^1 cfu/g. Coffee products had no *S. aureus* count.

TABLE 1: MEAN TOTAL MICROBIAL COUNTS (cfu/g) FOR COCOA, TEA AND COFFEE SAMPLES

Cocoa					Tea				Coffee		
SC	**TAPC**	**TFC**	**SAC**	**TCC**	**SC**	**TAPC**	**TFC**	**SAC**	**SC**	**TAPC**	**TFC**
C1	$1.2\times10^1\pm0.00$	$2.9\times10^1\pm0.00$	< 10	-	**T1**	$3.3\times10^2\pm0.03$	$3.6\times10^2\pm0.01$	$3.4\times10^1\pm0.00$	**CF1**	$4.5\times10^1\pm0.00$	$2.2\times10^1\pm0.00$
C2	$2.7\times10^2\pm0.02$	$3.0\times10^2\pm0.02$	$7.6\times10^1\pm0.00$	$3.2\times10^1\pm0.00$	**T2**	$3.0\times10^2\pm0.01$	$4.0\times10^2\pm0.01$	-	**CF2**	$5.5\times10^1\pm0.00$	$2.7\times10^1\pm0.00$
C3	$1.7\times10^1\pm0.02$	$2.8\times10^2\pm0.01$	< 10	$4.2\times10^1\pm0.00$	**T3**	$4.1\times10^2\pm0.01$	$3.5\times10^2\pm0.01$	-	**CF3**	$2.2\times10^1\pm0.00$	$2.8\times10^1\pm0.00$
C4	$1.3\times10^1\pm0.00$	$2.9\times10^1\pm0.00$	< 10	-	**T4**	$4.5\times10^2\pm0.02$	$4.6\times10^2\pm0.02$	$4.7\times10^1\pm0.00$	**CF4**	$1.0\times10^1\pm0.00$	$2.7\times10^1\pm0.00$
C5	$2.1\times10^2\pm0.01$	$2.2\times10^1\pm0.00$	< 10	-	**T5**	$3.5\times10^2\pm0.01$	$4.5\times10^2\pm0.01$	-	**CF5**	$2.2\times10^1\pm0.00$	$2.9\times10^1\pm0.00$
C6	$1.0\times10^1\pm0.00$	$3.4\times10^2\pm0.02$	< 10	-	**T6**	$2.8\times10^2\pm0.00$	$2.9\times10^2\pm0.01$	-			
C7	$2.9\times10^2\pm0.01$	$4.4\times10^2\pm0.03$	$1.1\times10^2\pm0.00$	$4.6\times10^1\pm0.01$	**T7**	$1.7\times10^1\pm0.00$	$2.8\times10^1\pm0.00$	-			
C8	$1.7\times10^2\pm0.00$	$3.6\times10^2\pm0.02$	$2.9\times10^1\pm0.01$	$2.6\times10^1\pm0.00$	**T8**	$2.9\times10^2\pm0.01$	$3.1\times10^2\pm0.00$	-			
C9	$2.6\times10^1\pm0.00$	$2.3\times10^1\pm0.00$	-	-	**T9**	$2.2\times10^2\pm0.01$	$2.6\times10^1\pm0.00$	-			
C10	$1.7\times10^1\pm0.00$	$3.8\times10^2\pm0.03$	-	$3.8\times10^2\pm0.01$	**T10**	$3.7\times10^1\pm0.00$	$2.7\times10^1\pm0.00$	< 10			

Key: SC= Sample code; TAPC= Total aerobic plate count; SAC= *S. aureus* count; TCC= Coliform count; TFC= Fungal count; C= Cocoa products; T= Tea products; CF= Coffee products; — = No Bacterial Growth

The table also reveals the presence of coliforms in some cocoa samples with counts ranging from 2.6×10^1 cfu/g in C8 to 4.6×10^1 cfu/g in C7. Tea and coffee samples were however not contaminated by coliforms. All the samples had fungal counts of the order 10^1 to 10^2. The cocoa and tea products, however, had more fungal contaminants when compared to the coffee samples though not significant p= 0.05. Microbial isolates documented were mainly species of *Bacillus* (59.2%), *Aspergillus* (20.0%) *and Penicillium* (14.4%) and pockets of *Saccharomyces cerevisae*

(12.0%), *Staphylococcus* (12.0%) and *Enterobacter* (1.6%).

Chemical evaluation

Tables 2, 3 and 4 present the proximate and mineral compositions of the samples. Cocoa samples (C7 and C10) had higher proximate parameters with the exception to carbohydrates. Tea and coffee products had low lipids (Ether extracts) except, however, for coffee sample CF4 with 3.67 % lipid. The tea samples have high moisture contents but for T7, T9 and T10. There is a weak positive correlation among the proximate and mineral values of cocoa, coffee and tea with significant values of 0.333, 0.117 and 0.054 respectively. Similarly, the result shows that proximate values and microbial counts are positively correlated with 0.329 and a significant relationship with 0.001 p-values. The association was stronger for TAPC and TFC to protein, ash and Mg components of cocoa products but not with tea and coffee All the samples contained phytochemicals (phenolics and flavonoids) and with antioxidant property (see Table 5).

TABLE 2: PERCENTAGE (%) PROXIMATE AND MINERAL (Mg/Kg) COMPOSITIONS FOR COCOA PRODUCTS

Sample	Moisture	Protein	Ether extract	Ash	Crude fiber	Carbo hydrate	Mg	Ca	Fe	Cu
C1	2.31±0.01	5.934±0.89	3.559±0.07	1.808±0.01	0.281±0.04	86.108±0.03	210.9±3.08	0.606±0.01	90.438±1.80	0.01±0.00
C2	1.72±0.01	7.601±0.12	5.227±0.02	1.481±0.01	0.805±0.06	83.176±0.05	208.5±1.79	0.330±0.02	4.292±1.60	0.37±0.00
C3	1.44±0.13	8.935±0.07	0.790±0.08	5.380±0.13	0.122±0.06	83.333±0.09	213.3±2.27	1.466±0.07	33.044±1.19	0.06±0.00
C4	2.22±0.07	15.385±0.04	5.841±0.02	4.583±0.04	0.123±0.04	71.848±0.42	216.4±3.66	0.876±0.08	6.9723±1.76	0.26±0.01
C5	2.34±0.75	16.827±2.90	4.385±0.07	4.461±0.21	0.750±0.01	71.237±0.79	211.9±2.85	0.204±0.07	3.529±1.21	0.24±0.02
C6	0.88±0.03	8.847±0.06	1.572±0.05	4.056±0.08	0.770±0.01	83.875±0.08	221.3±2.08	0.373±0.05	36.729±1.61	0.01±0.00
C7	6.89±0.04	25.115±0.11	8.527±0.09	9.025±0.04	0.152±0.05	50.291±0.60	230.4±1.60	1.118±0.05	865.94±4.82	15.01±0.14
C8	1.43±0.02	11.485±0.01	8.580±0.08	2.646±0.22	0.215±0.08	75.644±0.09	259.3±2.03	0.266±0.01	46.044±1.85	0.08±0.00
C9	2.25±0.11	9.026±0.01	2.497±0.05	3.884±0.30	0.206±0.07	82.137±0.12	213.1±4.31	0.178±0.04	11.492±1.81	0.27±0.01
C10	4.04±0.02	25.135±0.01	20.062±0.10	6.845±1.71	0.391±0.02	43.527±0.18	230.7±3.27	0.425±0.02	148.52±3.20	40.5±0.40

TABLE 3: PERCENTAGE (%) PROXIMATE AND MINERAL (Mg/Kg) COMPOSITIONS FOR TEA PRODUCTS

Sample	Moisture	Protein	Ether extract	Ash	Crude fiber	Carbo hydrate	Mg	Ca	Fe	Cu
T1	7.27±0.04	2.802±0.10	0.896±0.02	5.357±0.23	9.329±0.12	74.346±0.12	217.3±4.02	0.364±0.02	27.770±0.08	0.16±0.00
T2	8.11±0.07	2.747±0.05	0.684±0.05	4.772±0.05	11.013±0.42	72.674±0.13	199.5±2.37	0.400±0.01	4.360±0.06	0.18±0.01
T3	7.11±0.02	2.853±0.07	0.437±0.01	4.453±0.09	9.461±0.28	75.686±0.09	207.7±3.82	0.599±0.03	5.329±0.24	0.12±0.00
T4	8.13±0.06	2.641±0.08	0.868±0.06	2.909±0.22	8.049±0.16	77.403±0.12	205.6±3.47	0.480±0.00	12.677±0.29	0.44±0.02
T5	6.73±0.96	2.542±0.08	0.448±0.00	3.670±0.05	6.084±0.01	80.526±0.22	205.2±3.14	0.580±0.01	3.622±0.01	0.28±0.01
T6	10.00±0.05	2.488±0.02	0.499±0.01	3.848±0.14	12.260±0.33	70.905±0.11	207.2±3.06	0.678±0.03	33.885±0.09	0.60±0.02
T7	0.73±0.01	1.700±0.07	0.554±0.02	0.139±0.04	0.037±0.01	96.840±0.02	178.9±2.88	0.099±0.02	6.124±0.05	0.11±0.00
T8	7.77±0.01	2.952±0.01	0.585±0.01	4.221±0.04	9.420±0.08	75.011±0.30	208.5±3.11	0.340±0.01	10.529±0.93	0.02±0.00
T9	0.54±0.10	0.981±0.03	0.173±0.00	3.020±0.03	0.001±0.00	95.285±0.42	70.6±1.80	0.120±0.01	00.000±0.00	0.16±0.01
T10	1.50±0.01	1.015±0.14	0.239±0.01	2.014±0.02	0.005±0.00	95.200±0.04	81.9±2.10	0.240±0.04	0.065±0.01	0.01±0.00

TABLE 4: PERCENTAGE (%) PROXIMATE AND MINERAL (Mg/Kg) COMPOSITIONS FOR COFFEE PRODUCTS

Sample	Moisture	Protein	Ether extract	Ash	Crude fiber	Carbo hydrate	Mg	Ca	Fe	Cu
CF1	3.71±0.03	2.567±0.08	0.090±0.00	6.272±0.43	0.795±0.02	86.566±0.38	216.4±3.77	0.259±0.03	1.524±0.10	0.02±0.00
CF2	2.70±0.02	2.308±0.08	0.507±0.01	4.745±0.24	2.077±0.03	87.663±0.36	227.8±4.09	0.486±0.12	36.216±2.00	3.81±0.13
CF3	3.57±0.17	2.711±0.01	0.139±0.01	4.110±0.13	0.047±0.00	89.423±0.32	208.6±3.78	0.231±0.01	0.980±0.01	0.01±0.00
CF4	2.45±0.09	2.459±0.02	3.669±0.33	4.285±0.02	0.235±0.01	86.902±0.50	216.4±2.76	0.300±0.04	0.880±0.02	0.65±0.02
CF5	3.40±0.06	2.242±0.01	0.177±0.01	3.454±0.01	1.528±0.05	89.199±0.14	218.7±5.54	0.260±0.03	1.699±0.10	0.00±0.00

DISCUSSION

Microbiological analysis

All the products analyzed had TPC and fungal counts not significantly ($p<0.05$) different from the standard requirement as indicated in (29, 30, 31) these standards specify a TAPC of 10^2-10^4 cfu/g and fungal counts of 10^2-10^3 cfu/g sample.

TAPC (heterotrophic contaminants) if made up of mere environmental contaminants of no health concern, will pose no threat to consumers but for food spoilage. However, the presence of organisms of public health concern makes them unacceptable. Fungi are spore bearers that are known to be common environmental and food contaminants, their presence in these samples, therefore, corroborate earlier reports (32). There is abounding evidence, however, that although fungi are common food contaminants, some species of *Aspergillus, Penicillium, Fusarium* can produce deleterious toxins [12,] and have been implicated in life threatening food borne diseases (32).

The level of *S. aureus* count in the samples was low; however, it is advisable that *Staphylococcus aureus* should not be present in a refined food product. The presence of *Staphylococci* could be attributed to the human beings involved in the processing. *Staphylococci* are normal flora of man and have been reported as contaminants in foods and also implicated in foodborne illnesses (33, 34).

The FAM(29), TRISL(30), and FDA(31) specify total *Coliform* counts of not more than 10^1 cfu/g (1.8-10^1 cfu/g) for cocoa, coffee powder products and tea, some products analyzed in this work (C2, C3, C7, and C8) had significantly higher counts 2.6×10^1 cfu/g – 4.6×10^1 cfu/g. The coffee and tea products samples were negative for total coliform. Coliforms were indicator organisms; their presence in some products above acceptable limits connotes lapses in the quality control processes of manufacturing practices (35). This also portends a potential health hazard to consumers because these products are ready-to-eat.

The presence of bacillus spp as the major contaminants of products analyzed is in tandem with reports that bacilli are spore formers that are ubiquitously distributed. Some species *B. cereus, B. anthraces* have been implicated in foodborne diseases (36, 37), the presence of bacillus, therefore, must be closely monitored and not just be treated as mere contaminants.
All the products analyzed met the required microbiological standard, with the exception of some cocoa samples containing sub-standard coliform counts; this could indicate that most of the products were of good quality assuming all other organisms/metabolites of concern not assayed for were absent. The positive correlation established for proximate values and microbial counts of products corroborate reports that have shown cocoa powders and powdered cocoa beverages as good culture media for bacteria and fungi despite their low moisture content and powdery or granular nature (38, 19). Cocoa, tea and coffee products just like other food products can be contaminated by the food handlers and the environment with a wide variety of microorganisms during manufacturing and packaging processes. Thus if these products are not properly handled and stored in a hygienic environment, the shelf life could be reduced and the hygroscopic nature of these products could support the growth and proliferation of microbial contaminants to hazardous levels.

Chemical analysis
The proximate compositions of the samples were within standard specifications except for percentage moisture which is high in some cocoa and tea products and low ash contents in some tea samples. The high moisture of C7 and some tea products could account for its higher microbial load compared to other samples (39). The quality of initial raw materials, processing conditions, and handling practices have been reported to reflect product quality (40, 41), This could explain why some cocoa products (C2, C3, and C8) with low moisture contents have relatively high contaminants level while some tea products (T2, T3, T5, T6, T8) with high moisture contents tend to have low microbial loads. Although all the products except for the once earlier mentioned, had low moisture level that might not constitute suitable medium for the growth of microbes, poor storage conditions, and increase in moisture has been identified as factors that could affect products shelf life, favour microbial growth, proliferation and subsequent production of toxic microbial metabolites such as mycotoxins (39).

The mineral compositions as reported for cocoa samples are in consonance with the presentations of Jayeola and Oluwadun(19). All the samples had copper compositions within standard required limit of 30 mg/kg, except for sample C10 with a copper level of 40.5 mg/kg which is within the SLTB maximum acceptable limit of 100 mg/kg. Copper is a heavy metal and copper toxicity is allied with stomach upset, queasiness, and diarrhea and can front tissue injury and disease (42). At high concentrations, copper is known to produce oxidative damage to biological systems, including peroxidation of lipids or other macromolecules (43, 44). Copper, however, is an

essential trace mineral for man, it plays an important role in diseases in which oxidant stress is elevated. Deficiency of copper has been observed to alter the role of other cellular constituents involved in antioxidant activities, such as iron, selenium, and glutathione (45, 46, 47).

The samples all contained some levels of phytochemicals; beverages (coffee, tea, and cocoa) have been reported to contain polyphenols and minerals that have diverse beneficial biochemical, antioxidant and antimicrobial effects (48, 49, 21). Tea contributes to approximately 63 % of dietary flavonoid in the diet, and 69–85 % of the flavonoid content is soluble in hot water brewing within the first 3–5 min. (50,51) opined that the phenols and phytochemicals content in beverages are a function of the starting materials, roasting levels, and brewing method. Free radical scavenging assay is used to investigate the antioxidant capacity/ activity of foods (52, 53). The products analyzed exhibited different levels of antioxidant activity. Many studies have considered fruits, vegetables, and teas as the major sources of dietary antioxidative phenolics, this report observed cocoa product CI exhibited high scavenging activity (high antioxidant level with lowest IC_{50}) against DPPH free radicals. Arts *et al*,(54) reported that the antioxidant catechin content of chocolate (cocoa product) is four times that of tea.

In conclusion, some of the cocoa and tea products failed the required standard for coliform and moisture contents; this could be due to poor manufacturing practice and storage, it was observed in this study that some tea and cocoa products which did not meet the required standards are products produced and consumed locally in Nigeria. Use of quality raw materials, efficient and good manufacturing practice (GMP), proper storage conditions, application of hazard analysis critical control points (HACCP) should be well implemented and enforced by regulatory agencies. Quality products with good antioxidant activity should be encouraged for the health benefits.

Conflict of interest: Both authors declare no conflict of interest and have approved the submission of this article to this journal

Significance Statement: This study discovers that Cocoa, Coffee & Tea beverages at consumer level do contain microbes including coliforms that can cause foodborne infection/intoxication. A positive relationship was established for microbial load, proximate compositions and mineral values in addition to the antioxidant property of these beverages. This study will help the researcher to uncover the critical area of beverages in functional foods, as a carrier medium for probiotics and fortification for better antioxidant activity. The need for strict adherence to good manufacturing practices and hazard analysis critical control point in beverage production is emphasized.

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REFERENCES

1. Andrew, H., 2008. Microbiological risks associated with hot-drinks vending. PhD Thesis, University of Wales. https://repository.cardiffmet.ac.uk/bitstream/handle/10369/860/ Andrew%20Hall%20-%20PhD%20thesis.pdf?sequence=1

2. Tournas, V.H. and Katsoudas, E.J., 2008. Microbiological quality of various medicinal herbal teas and coffee substitutes. Microbiolo. Insights, 1: 47–55. http://insights.sagepub. com/microbiological-quality-of-various-medicinal-herbal-teas-and-coffee-su-article-a1038-abstract?

3. Alan, C., Hiroshi, A. and Francisco, T.B., 2011. Teas, Cocoa and Coffee: Plant secondary metabolites and health. Wiley-Blackwell ISBN: 978-1-4443-3441-8, 264 Pp. http://ubblab.weebly.com/uploads/4/7/4/6/47469791/teas,_cocoa_and_coffee.pdf

4. Angus, D., 1999. Commodity prices and growth in Africa. J. Econ. Pers.,13(3): 23–40. https://www.princeton.edu/~deaton/downloads/Commodity_Prices_and_Growth_in_Africa.pdf

5. Lei, W.U., 2014. Cash crops and food security: Contributions to income, livelihood risk and agricultural innovation. LEI Wageningen UR, April 2014 report. Dutch Ministry of Economic Affairs 'Food security' research (project number BO-20-007-004). https://www.wur.nl/en/newsarticle/Cash-crops-enhance-food-security.htm

6. Claire, S.C., 2014. Agricultural value chains in Sub-Saharan Africa from a development challenge to a business opportunity. Deutsche Bank Research, 60262 Frankfurt am Main, Germany.

7. Bhat, R., Rai, R.V. and Karim, A.A., 2010. Mycotoxins in food and feed: present status and future concerns. Comp. Rev. Food Sci. Food Safety, 9:57-81. DOI:10.1111/j.1541-4337.2009.00094.x

8. Cenacle research, 2014. Food borne disease outbreak analysis. http://www.slideshare.net/KrishnaPG/food-borne-disease-outbreak-analysis-by-cenacle-research. Accessed 29/12/16

9. Abdel-Hafez, A. and El-Maghraby, O.M., 1992. Fungal flora and Aflatoxin associated with cocoa, roasted coffee and tea powders in Egypt. Cryptogamie Mycologie, 13: 31-45.

10. Nakajima, M., Tsubouchi, H., Miyabe, M. and Ueno, Y., 1997. Survey of aflatoxin B1 and ochratoxin A in commercial green coffee beans by high-performance liquid chromatography linked with immunoaffinity chromatography. Food Agr. Immunol., 9: 77–83.

11. Batista, L.R., Chalfoun, S.M., Prado, G., Schwan, R.F. and Wheals, A.E., 2003. Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica L.*). Int. J. Food Microbiol., 85: 293–300.

12. Durand, N., El-Sheikh, M.A., Suarez-Quiroz, M.L., Gonzales-Rios, O., Nganou-Donkeng, N., Fontana-Tachon, A. and Montet, D., 2013. Application of PCR-DGGE to the study of dynamics and biodiversity of yeasts and potentially OTA producing fungi during coffee processing. Food Cont., 34 (2): 466-471.

13. Lawley, R., 2013. Ochratoxins. Food Safety Watch. http://www.foodsafetywatch.org/factsheets/ochratoxins/Accessed 29/12/16

14. Mounjouenpou, P., Durand, N., Guiraud, J.P., Maboune-Tetmoun, S.A., Gueule, D. and Guyot, B., 2013. Assessment of exposure to ochratoxin - A (OTA) through ground roasted coffee in two cameroonian cities: Yaounde and Douala. Intern. J. Food Sci. Nut. Eng.,3 (3): 35-39. http://dx.doi.org/10.5923/j.food.20130303.03

15. Malinowska, E., Inkielewicz, I., Czarnowski, W. and Szefer, P., 2008. Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. Food Chem. Toxicol., 46 (3): 1055–61. DOI:10.1016/j.fct.2007.10.039.

16. Lung, S.C., Cheng, H.W. and Fu, C.B., 2008. Potential exposure and risk of fluoride intakes from tea drinks produced in Taiwan. J. Exp. Sci. Env. Epidemiol., 18(2): 158–66. DOI:10.1038/sj.jes.7500574.https://www.wur.nl/en/newsarticle/Cash-crops-enhance-food- security.htm

17. Schwalfenberg, G., Genuis, S.J. and Rodushkin, I., 2013. The benefits and risks of consuming brewed Tea: Beware of toxic element contamination. J. Toxicol., 2013: 1-8. ID 370460, http://dx.doi.org/10.1155/2013/370460

18. Food and Agriculture Organization of United Nations (FAO), 2006. Guidelines for the prevention of mould formation in Coffee. Available at: ftp://ftp.fao.org/ag/agn/ coffee/ guidelines_final_en.pdf. Accessed on 03/01/2017.

19. Jayeola, C.O. and Oluwadun, A., 2010. Mycoflora and nutritional components of cocoa powder samples in Southwest Nigeria. Afr. J. Agr. Res., 5: 2694 2698. http://www. academicjournals.org/journal/AJAR/article-full-text-pdf/BC7AAD738278

20. Nurk, E., Refsum, H., Drevon, C.A., Tell, G.S., Nygaard, H.A., Engedal, K. and Smit, A.D., 2009. Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance1–3. J. Nut., 139: 120–127. Doi: 10.3945/jn.108.095182.

21. Suarez-Quiroz, M.L., Taillefer, W., López-Méndez, E.M., Gonzalez-Rios, O., Villeneuve, P. and Figueroa-Espinoza, M.C., 2013. Antibacterial activity and antifungal and anti-mycotoxigenic activities against *Aspergillus flavus* and *A. ochraceus* of green coffee chlorogenic acids and dodecyl chlorogenates. J. Food Safety, 33 (3): 360-368.

22. Wierzejska, R., 2014. Tea and health – a review of the current state of knowledge. Przegl. Epidemiol., 68: 501 – 506.

23. Speck, M.L., 1976. Compendium of methods for microbiological examination of foods, American Public Health Association. Washington DC pp 277 – 328.https://fbns.ncsu.edu/USDAARS/Acrobatpubs/P151-189/p180.pdf

24. Jolt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T. and Williams, S.T., 1994. Bergey's manual of systematic bacteriology, 9th edn. Williams & Wilkins Co. Baltimore, Maryland, p. 786.

25. Tsuneo, W., 2010. Pictorial atlas of soil and seed fungi: Morphologies of cultural fungi and key to species. Third edition CRC press 2010.

26. AOAC, 2008. Association of Official Analytical Chemists. Official Methods of Analysis (18th Ed.), AOAC, Arlington, USA.

27. Oranusi, S., Nwachukwu, C., Adekeye, B.T., Dahunsi, O.S. and Adeyemi, A.O., 2013. Microbial profile, antibacterial and antioxidant activities of some imported spices in Nigeria. Eur. J. Exp. Biol., 3(6):193-202.

28. Brand-Williams W., Cuvelier, M.E. and Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm.Wiss.Technol., 28: 25-30. http://radio.cuci.udg.mx/bch/EN/Manuals/Techniques/DPPH-original_LebensWissTechnol_1995-v28-p25.pdf

29. Food Administration Manual (FAM), 1995.Microbiological reference criteria for food *version* 2.0, 15-26Pp.

30. Tea Research Institute of Sri Lanka (TRISL), 2011.Standards of tea for ensuring market requirement.

www.tri.lk/userfiles/file/223_E&E/3_223_E&E_Presenta.

31. FDA, 2013. Revised guidelines for the assessment of microbiological quality of processed foods. FDA Philippines Circular No. 2013-010 Pp 1-11.

32. Oranusi, S. and Dahunsi, S.O., 2015. Preliminary study on hazards and critical control points of kokoro, a Nigerian indigenous fermented maize snack. *SpringerPlus* 4:253. DOI 10.1186/s40064-015-1026-3.

33. Hennekinne, J., De-Buyser, M. and Dragacci, S., 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol. Rev. 36:815–836. DOI:10.1111/j.1574-6976.2011.00311.x

34. Ji-Yeon, H., Chang, G,. Bing, S., Kwon, K., Lee, H. and Kim, S., 2013. A foodborne outbreak of *Staphylococcus aureus* associated with fried chicken in Republic of Korea.J. Microbiol. Biotech., 23:85–87.

35. Ingram, M. and Roberts, T.A., 1976. The Microbiology of the red meat carcass and the slaughter house. R. Soc. Health J., 96(6):270-6.

36. Lesley, M.B., Velnetti, L., Yousr, A.N., Kasing, A. and Samuel, L., 2013. Presence of *Bacillus cereus* from ready-to-eat cereals (RTE) products in Sarawak. Int. Food Res. J., 20(2):1031–1034.

37. Aruwa, C.E. and Akinyosoye, F.A. 2015. Microbiological assessment of ready-to-eat foods (RTEs) for the presence *Bacillus* species. J. Adv. Biol. Biotechnol.,3(4): 145-152.http://www.journalrepository.org/media/journals/JABB_39/2015/May/Aruwa342015JABB17407.pdf

38. Adesina, F.C., Fagade, O.E. and Ogunjobi, A.A., 2010. Microbiological quality of sachet packed cocoa based beverages marketed in Ibadan, Nigeria. J. Appl. Biosci., 33: 2070 –2075.

39. Mendonca, J. C. F., Franca, A.S. and Oliveira, L. S., 2007. A comparative evaluation of methodologies for water content determination in green coffee. LWT 40: 1300–1303.

40. Carbonell-Barrachina, A.A., García, E,. Sánchez, S.J., Aracil, P. and Burló, F., 2002. Effects of raw materials, ingredients, and production lines on arsenic and copper concentrations in confectionery products. J. Agric Food Chem., 50(13):3738-42.

41. Amsbary, R., 2013. Raw materials: Selection, specifications, and certificate of analysis. Quality Assurance & Food Safety-http://www.qualityassurancemag.com/magazine/ Accessed 17/02/2017.

42. Higdon, J., Drake, V.J., Delage, B. and Prohaska, J.R., 2017. Copper. Linus Pauling Institute Micronutrient Information Center, Oregon State University. http://lpi. oregonstate.edu/mic/minerals/copper. Accessed 17/02/2017

43. *Bremner, I., 1998. Manifestations of copper excess. The Am. J. Clin. Nut., 67(5 Suppl): 1069S–1073S.*

44. Ralph, A. and McArdle, H.J., 2001. Copper metabolism and requirements in the pregnant mother, her fetus, and children. International Copper Association, New York.

45. *Kaegi, J.H.R. and Schaeffer, A., 1988. Biochemistry of metallothionein. Biochemistry.27 (23): 8509–15. DOI:10.1021/bi00423a001.*

46. *Salem, H., Green, S., Bigelow, S.,. Borzelleca, J. and Baskin, S,.1992. Preface. Critical Rev. Food Sci. Nut.,32 (1): 1–31. Doi:10.1080/10408399209527583.*

47. *Stern, B.R. 2007. U-Shaped dose-response curve for risk assessment of essential trace elements: Copper as a Case Study. In Robson MG, Toscano WA (eds), Risk assessment for environmental health. San Francisco: John Wiley and Sons. 555–62 Pp.*

48. Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C.L., Hollenberg, N.K., Sies, H., Kwik-Uribe, C., Schmitz, H.H. and Kelm, M., 2006. (_)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. PNAS103(4):1024–1029. www.pnas.org_cgi_doi_10.1073_pnas.0510168103

49. Buijsse, B., Feskens, E.J., Kok, F.J. and Kromhout, D., 2006. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch. Intern. Med.,166(4): 411-417. DOI:10.1001/archinte.166.4.411

50. Keli, S.O., Hertog, M.G.L., Feskens, E.J.M. and Kromhout, D., 1996. Dietary flavonoids, antioxidant vitamins, and incidence of stroke.Arc. Int. Med. 156: 637–642.

51. Ferruzi, M., 2010. The influence of coffee and tea beverage composition on delivery of phenolics. Physiol. Behav., 100(1): 33-41. DOI:10.1016/j.physbeh.2010.01.035

52. Kim, D.O., Lee, K.W., Lee, H.J. and Lee, C.Y., 2002.Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J. Agr. Food Chem.,50: 3713-3717. DOI: 10.1021/jf020071c

53. Asimi, O.A., Sahu, N.P. and Pal, A.K., 2013. Antioxidant activity and antimicrobial property of some Indian spices. Intern. J. Sci. Res. Pub., 3(3): 1-8. http://www.ijsrp. org/research-paper-0313/ijsrp-p15110.pdf

54. Arts, I.C., Hollman, P.C. and Kromhout, D., 1999. Chocolate as a source of tea flavonoids. Lancet 354, 488. DOI:10.1016/S0140-6736(99)02267-9