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Proximate Composition and Aflatoxin Composition of Retailed Cocoa Powdered Beverage

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Abstract
The cocoa tree (*Theobroma cacao* L.), belonging to the Malvaceae family is a tropical plant cultivated for its beans. Its derived products such as cocoa powder, cocoa liquor and chocolate are a very rich source of bioactive components. However, cocoa beans are usually susceptible to fungi contamination during certain stages of processing on the farm. In this study, eleven samples of cocoa powder were purchased in Ibadan and taken to the laboratory for determination of proximate and aflatoxin content. The proximate composition of the samples was determined using the analytical methods adopted by the Association of official analytical chemists (AOAC) while aflatoxin contents were done using the enzyme-linked immunosorbent assay (ELIZA). The proximate determination showed that the moisture contents of the samples ranged from 4.3819.82%, ash contents ranged from 4.87-11.32%, crude fat ranged from 3.22-14.15%, oil contents ranged from 2.1-4.55 while carbohydrate contents ranged from 59.87-80.28. The aflatoxin analysis of the cocoa powder in part per billion ranged from 10-55. Conclusively, the proximate composition of the cocoa powder justifies its claim that it is highly rich in nutrients but highly shown to be contaminated with aflatoxins which pose serious health risk to its consumers.

Keywords: Cocoa powder, aflatoxin, ELIZA, proximate, crude fat

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Introduction

The genus *Theobroma* of which *Theobroma cacao* is a specie originated from Amazon and Orinoco basins and later spread to the regions of South America, particularly, Mexico. The Olmec and Mayas later termed "the drinks of gods". Although *Theobroma* has 22 species the most common is the *Theobroma cacao* (Asiedu, 2017). The cocoa tree (*Theobroma cacao L.*), the Malvaceae family is a tropical plant cultivated for its beans which are extracted powder and cocoa butter. Cocoa is an important source of income for small farmers, who are responsible for the bulk of world production (Adama *et al*., 2013).

Cocoa is an important crop around the world: it is an important cash crop for growing countries, as well as an important branch of the food industry for processing and consuming countries.

Nigeria is the third largest producer of Cocoa in West Africa. While the crop is sometimes farmed on a large scale, the sector is dominated by small scale farmers and remains a critical source of livelihood for rural populations in states where the crop is produced (CPPA, 2017). In the South-west, cocoa-producing states include Ondo, Oyo, Osun Ogun and Ekiti where farmers either operate on inherited field or operate a share

cropping system in which two-thirds of the produce accrues to the land owner who also contributes to purchase of farming input (CPPA, 2017).

Cocoa and its derived products (cocoa powder, cocoa liquor, and chocolate) are a very rich source of bioactive components such as polyphenols. The main groups of cocoa polyphenols are catechins (37%), anthocyanins (4%), and proantocyanidins (58%). The amounts of polyphenols vary due to the type of cultivar and the country of origin, as well as production processes of cocoa beans and chocolate manufacturing processes.

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 sportsmen and women (Olugbuyiro *et al.,* 2011). 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 a age-related health problems, fatigue, hypertension, diabetes, breast cancer, cardiovascular and mental diseases (Olubamiwa, 2007). Cocoa beans can become contaminated by fungi during pre-processing at the farm, especially during drying or storage and some fungal species can produce mycotoxins when growing in foods.

Mycotoxins are toxic secondary metabolites produced by fungi in agricultural products that are susceptible to mould infestation and can be classified according to their fungal origin, chemical structure and biological activity (Sowley, 2016). They are commonly produced by fungi belonging to the genera, *Aspergillus*, *Fusarium* and *Penicillium.* Mycotoxin production and contamination are unavoidable and depend on a variety of environmental factors in the field and or during storage, which makes it a unique challenge to food safety. Their occurrence in food is mainly as a result of direct contamination of agricultural commodity and their survival of food processing to some extent. Over 200 mycotoxins have been reported but only those occurring naturally in foods are of significance in food safety (Sowley, 2016).

Aflatoxins are one of the naturally occurring mycotoxins that are found in soils, foods, humans, and animals. They are derived from the *Aspergillus flavus* fungus, the toxigenic strains of aflatoxins are among the most harmful mycotoxins. Aflatoxins are found in the soil as well as in grains, nuts, dairy products, tea, spices and cocoa, as well as animal and fish feed. Aflatoxins are especially problematic in hot, dry climates (+/- 30 to 40 degrees latitude) and their prevalence is exacerbated by drought, pests, delayed harvest, insufficient drying and poor post-harvest handling. Exposure to foods contaminated with high levels of aflatoxins can cause immediate death to humans and animals. Chronic high levels lead to a gradual deterioration of health through liver damage and immune suppression (Negesh, 2018). Aflatoxin contamination occurs either in the field or during storage between the temperatures of 20-40 ºC, having 10-20% humidity and with 70-90% relative humidity in the air. The *Aspergillus* species normally grow at a temperature range of 8-55 ºC with the optimal temperatures ranging from 36-38 ºC and 25-35ºC actively supporting aflatoxin production. Aflatoxins production is inhibited at temperatures below 10 ºC and above 45 ºC (Asiedu 2017). The health conditions caused by aflatoxins are varied and depend on the level and length of exposure. Some of the health problems posed by aflatoxin contamination include aflatoxicosis, cancer, infertility, hepatocellular carcinoma, liver cirrhosis, nephropathy, immunodeficiency, anaemia, stunting, underweight in humans and nutritional interference (Sowley, 2016) 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 sportsmen and women. This recent increase in the consumption of cocoa food products has been ascribed to the ongoing awareness campaign that has shown cocoa to prevent age related health problems, fatigue, hypertension, diabetes, breast cancer,

cardiovascular and mental diseases. However cocoa beans a major raw material for various cocoa products most often are susceptible to fungi contamination during certain stages of processing on the farm thus leading to contamination of cocoa products with mycotoxins which has made cocoa product such as cocoa beverages susceptible to mycotoxins contamination. Hence the need to analyse the fungal, proximate and aflatoxin contents of selected cocoa beverages.

Materials and Methods

Collection of samples

A total of eleven (11) samples of cocoa powder were randomly purchased from markets in Ibadan Oyo state Nigeria.

Moisture content determination

This was done by the gravimetric method described by the AOAC (1990). A measured weight of the sample (5.0 g) was weighed into a previously weighed moisture can. The sample in the can was dried in the oven at 105°C for 3 h.

It was cooled in a dessicator and weighed. It was then returned to the oven for further drying. Drying, cooling and weighing were done repeatedly at hourly intervals until there were no further diminutions in the weight (that is, constant weight was obtained). The weight of moisture lost was calculated and expressed as a percentage of the weight of the sample analyzed. It was given by the expression below:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = Weight of empty moisture can

W_2 = Weight of empty can + Sample before drying

W_3 = Weight of can + Sample dried to constant weight

Determination of total ash content: This was done by the furnaces incineration gravimetric method described AOAC (1984). Briefly 5.0 g of the processed sample was measured into a previously weighed porcelain crucible. The sample was burnt to ashes in a muffle furnace at 550°C. When it has become completely ashed, it was cooled in desiccator and weighed.

The weight of ash obtained was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

$$\text{Ash (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W_1 = Weight (g) of empty crucible

W_2 = Weight of crucible + Ash

Determination of crude fat: This was determined by solvent extraction gravimetric method. Five grams of sample was wrapped in porous paper (whatman filter paper) and put in a thimble. The thimble was put in a soxhlet reflux flask and mounted into a weighted extraction flask containing 200 mL of petroleum ether. The upper of the reflux flask was connected to a water condenser. The solvent (petroleum ether) was heated, boiled vaporized and condensed into the reflux flask filled. Soon the sample in the thimble was covered with the solvent until the reflux flask filled up and siphoned over, carrying its oil extract down to the boiling flask. This process was allowed to go on repeatedly for 4 h before the defatted sample was removed, the solvent recovered and the oil extract was left in the flask. The flask (containing the oil extract) was dried in the oven at 60°C for 30 min to remove any residual solvent. It was cooled in desiccator and weighed. The weight of oil (fat) extract was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

$$\text{Fat (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W_1 = Weight (g) of empty extraction flask

W_2 = Weight of flask + oil (fat) extract

Protein determination

The total nitrogen was determined and multiplied with factor 6.25 to obtain protein content. Sample (0.5 g) was mixed with 10 mL of concentrated H_2SO_4 in digestion flask. A tablet of selenium catalyst was added to it before it was heated under a fume cup board until a clear solution was obtained (the digest). The digest was diluted to 100 mL in a volumetric flask and used for the analysis. The 10 mL of the digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distillation apparatus. The mixture was distilled into 10 mL of 40% boric acid containing 3 drops of mixed indicator (bromo cressol green/methyl red). A total of 50 mL of distillates was collected and titrated against 0.02 N EDTA from green to a deep red end point. A reagent blank was also digested, distilled and titrated. The nitrogen content and hence the protein content was calculated using the formula below:

1 mL of 1 N H_2SO_4 = 14 mg

Protein (%) = N_2 (%) x 6.25

$$N_2\,(\%) = \frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va} \times T.B$$

W = Weight of sample (0.5 g)
N = Normality of titrant (0.02 N H_2SO_4)
Vt = Total digest volume (100 mL)
Va = Volume of digest analyzed (10 mL)
T = Sample titre value
B = Blank titre value

Aflatoxin analysis

About 20ml of the samples was vigorously mixed with 100ml of 70/30v/v methanol/water and then filtered. The filtrate was dispensed into the dilution wells of the kit. About 100ul was transferred from the dilution wells into antibody-coated wells incubated at room temperature for 15mins. The microwells strips were washed and the strip was read with ELIZA reader using 450nm filter and 630nm differential filter.

Results

Table 1: proximate composition of selected Cocoa Powders

S/N	% Moisture	% Ash	% crude fat	Crude protein	Carbohydrate
C1	8.66	9.49	13.5	2.65	65.7
C2	7.8	6.88	14.15	2.1	69.7
C3	8.54	8.18	12.95	2.8	67.53
C4	5.4	10.58	10.95	2.62	70.45
C5	4.38	9.99	3.22	2.1	80.28
C6	7.94	11.32	11	3.32	66.42
C7	6.06	8.67	10.4	2.27	72.6
C8	5.98	7.79	11.2	4.02	72.01
C9	5.44	4.87	24.9	4.55	60.24
C10	19.82	7.89	10.3	2.18	59.87
C11	9.42	8.96	11.8	2.45	67.37

Table 2: Aflatoxin Content of the Cocoa Powder (ppb)

Samples	C1	C2	C3	C4	C5	C6	C7	C8
Aflatoxin content (ppb)	10	55	35	35	48	37	55	30

Discussion

Table 1 shows the result obtained from the proximate composition of sampled cocoa powders. The moisture content ranged from 4.38 to 19.82%. The moisture content values were much higher than the maximum limit of 5% specified for cocoa powders. These values obtained could be as a result of the fact that cocoa powder is hygroscopic and hence good packaging and storage conditions are essential to prevent take up of moisture (Adeyeye, 2016). The result obtained for the moisture is in contrast with values reported by Jayeola and Oluwadun, (2010) whose values ranged from 0.80-2.5%.

The ash content of the samples ranged from 4.87-10.58%. Ash is an indication of mineral contents of food and has been shown to be high in cocoa powder (Ndife *et al*., 2013). The crude fat ranged from 3.22 to 14.15%. Fat is important as it contributes to the overall energy value of foods. Adeyeye (2016) reported that most commercially available cocoa powder contain fat between 10-24%.

The crude protein ranged from 2.1-4.55% the protein values obtained in this work were lower to values reported by Ndife *et al*. (2013) and Jayeola and Oluwadun (2010). The carbohydrate content ranged from 59.87 to 72.6. The values are very high in all samples as this indicate that cocoa powder is a rich source of carbohydrates and energy which makes it desirable especially by the growing and young adult.

Generally, the variation in fat, protein and carbohydrates of the samples results from the different roasting temperatures, the method used in the extraction and the alkali treatment employed on the cocoa samples.

Table 2 shows the aflatoxin content of the cocoa samples, the aflatoxin content ranged from 10-55ppb. The high levels of aflatoxins in the samples may be attributed to the prior contamination of the cocoa beans used in the production of the cocoa powders or may be due to the resultant effect of the hygroscopic nature of cocoa powder thus leading to contaminant by aflatoxin producing moulds.

Conclusion

Aflatoxins contamination of the cocoa powders poses a serious threat to the safety of its consumers hence Control of mycotoxin is a matter of importance not only for health implications but also for improvement of the economy in the country. Several strategies for the reduction and control of mycotoxins such as prevention of mould growth in crops and other feedstuffs, decontamination of mycotoxin-contaminated foods and continuous surveillance of mycotoxins in agricultural crops, animal feedstuffs and human food should be enforced by the government on farmers and industries.

References

A.O.A.C. *(*1980). Official Methods of Analysis, 13th ed. Association of Official Analytical. Chemists. Washington D.C. 376-384

Adeyeye E.I (2016): Proximate, Mineral and Antinutrient Compositions of Natural Cocoa Cake, Cocoa Liquor and Alkalized Cocoa Powders Sourced in Nigeria. *Journal of Advanced Pharmaceutical Science and Technology* 1(3): 20-28

Asiedu, M. (2017). Levels of Ochratoxin A and Aflatoxins in cocoa beans from four cocoa growing regions in Ghana. A thesis submitted to the Department of food science and technology in partial fulfilment of the requirement of the award of the degree of Masters of Science.

Jayeola, C. O. and Oluwadun A. O (2010). Mycoflora and nutritional components of cocoa powder samples in South-West Nigeria. *African Journal of Agricultural Research*, 5(19): 2694-2698.

Ndife, J., Bolaji P., Atoyebi D. and Umezuruike C. (2013). Production and quality evaluation of cocoa products (plain cocoa powder and chocolate). *American Journal of Food and Nutrition.* 3(1): 31-38

Negash D. (2018). A review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. *J Nutr Health Food Eng*. 8(2):190 – 197

Olubamiwa O. (2017). *Have you had your cocoa today*? In National Cocoa

Development Committee. Feyisetan Press, Ibadan. pp 1-17.

Olugbuyiro A. O, Olasehinde G. I., McLoone, P. and Afolabi O. (2011). Relationship between Viable Bacterial Counts and Physicochemical Properties of Cocoa Powders and Powdered Cocoa Beverages purchased in Nigerian Supermarkets. *Researcher*, 3(3): 46 - 52

Sowley E. (2016). Aflatoxins: A silent threat in developing countries. *African Journal of Biotechnology* 15(35): 1864-1870