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Full Length Research Paper

Mycoflora and nutritional components of cocoa powder samples in South West Nigeria

Jayeola C. O.[1]* and Oluwadun A. O[2]

[1]Crop Processing and Utilization Division, Cocoa Research Institute of Nigeria, P.M.B., 5244, Ibadan, Oyo State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University Ago-Iwoye, Ogun State, Nigeria.

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The production of cocoa powder (CP), the major ingredient of cocoa-based beverages, has been on the increasing trend in Nigeria without much concern for whether or not they meet the microbiological criteria for food safety. This study was, carried out to investigate the mycroflora and intrinsic factors of twenty four brands of cocoa powder samples bought from different sources in South-West Nigeria with a view to determining their food safety and how their intrinsic factors affect microbial growth. A total of 360 samples of 24 brands of CP were purchased between April and November, 2007. The viable bacteria and mould counts were determined using standard plate count while the microbial isolates were identified using cultural, microscopic and biochemical methods. The pH, proximate, mineral and physical parameters were determined using recommended standard methods by Association of Official Analytical Chemists (AOAC). Student t-test and multiple linear regressions were employed in the statistical analysis of the data. The result showed variation in percentage of fat, protein and carbohydrate content as well as pH values from one CP to another. The pH values ranged from 6.4 to 7.4 while the moisture content of the CP was between 0.80 and 1.86%. The CP samples were found to be rich in magnesium, iron, sodium, potassium and carotenoids but deficient in vitamins. The common fungi isolated were *Aspergillus niger*, *Saccharomyces cerevisae*, *Penicillium chrysogenum* while the least encountered fungi were *Aspergillus melleus* and *Aspergillus chraceouso*. The study showed that CP, which forms the bulk ingredient of cocoa-based beverages, is a possible source of microbial contaminant to the beverages. The result also showed that the CP samples examined were rich in minerals and nutrients which could account for the survival of fungi in the samples.

Key words: Cocoa powder, microflora, beverages, contaminants.

INTRODUCTION

The mycoflora and the intrinsic factors that might contribute to the spoilage or deterioration of the cocoa powder are very important in order to ensure food safety. Consumption of cocoa powder (CP) is fast gaining ground in Nigeria due to its benefit in preventing age related diseases. Researches had shown that consumption of foods rich in polyphenolic compound may reduce the risk of cardiovascular diseases (Ding et al., 2006; Hollenberg, 2006). Cocoa contains flavonol which is a sub class of polyphenols that has been shown to prevent age related health problems, promotion of better cardiovascular and mental health as well as facilitating the treatment of many disease conditions (Grassi et al., 2006; Ottaviani et al., 2006). The merit cocoa consumption has over other crops rich in polyphenolic compounds is that, it contains abundant oligomeric procyanidins which is a bigger flavanol but, absent in others (Lazarus et al., 1999). Cocoa powder has a reduced water activity that may not constitute suitable substrate for the growth of microbes, but if not handled in hygienic form before consumption can result in the production of pathogenic organisms or production of toxic metabolites that can cause serious health problems.

Moulds are frequently found in cocoa beans and it is not uncommon to find mycotoxin-producing moulds and occasionally low levels of mycotoxins in cocoa. However,

*Corresponding author. E-mail: yinktay@yahoo.com.

Table 1. Proximate analysis of cocoa powder brands.

NO	Code No.	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	CHO (%)	Energy (cal/g)
1.	CIL	1.10NS	11.21b	20.85c	5.10b	58.74b	502.65
2.	FRL	1.44	10.50c	20.10c	6.22a	59.74a	413.86
3.	IOCP	1.12	12.01a	18.92d	4.88c	60.01a	424.05
4.	MNL	1.78	10.21c	22.01a	5.84b	58.16bc	412.57
5.	ONP	1.35	10.25c	22.54a	5.55b	58.31b	415.65
6.	SCP	1.26	11.12b	21.50b	4.26c	58.86b	421.52
7.	SF	1.65	10.25c	20.10c	6.45a	58.55bc	406.85
8.	FCP	0.84	11.50b	22.10a	6.15a	56.41cd	417.54
9.	PNL	2.51	10.45c	20.80c	5.24b	58.00c	409.25
10.	MTIL	1.66	10.33c	21.25b	4.68bc	59.08a	414.29
11.	NNP	1.15	11.43b	21.10b	5.40b	57.92c	418.95
12.	CNL	1.22	11.50b	20.55c	6.10a	56.63d	410.61
13.	NBC	1.68	11.01b	21.25b	5.25b	56.81d	411.33
14.	EFCO	1.30	12.72a	20.95c	5.15b	56.88d	425.80
15.	CCP	1.68	10.45c	21.00b	4.90b\	58.97b	413.93
16.	BAAK	1.45	11.40b	20.10c	4.26c	56.29d	408.16
17.	SUMAL	1.62	11.25b	21.15b	4.92bc	57.06c	414.09
18.	CRIN	0.92	10.40c	22.10a	5.28ab	58.50b	416.00
19.	L1	1.86	12.50a	21.22b	6.40a	54.02c	413.46
20.	L2	1.45	11.55b	20.20c	4.90bc	55.20c	402.85
21.	L3	1.84	10.20c	22.01a	5.29ab	55.66c	402.48
22.	L4	1.32	12.10a	21.01b	6.02a	54.55f	369.12
23.	L5	1.92	11.50ab	21.25b	5.50b	54.66f	410.68
24.	GCP	0.80	11.56ab	22.00a	5.96a	58.87b	427.48

Values followed by the same alphabet in the same column are not significantly different at $p < 0.05$. NS = Not significantly different. Codes 1 - 24 represent the different processing companies in Nigeria. Full names are available on request.

raw cocoa beans do not readily support the production of mycotoxins although they do support abundant growth of mycotoxin-producing fungi (Llewellyn et al., 1978). Beside *Aspergillus* being among the fungus genera, it has also been implicated in mycotoxicosis because it produces toxic metabolite called mycotoxins in food. Some of the species of this genus that have been severally reported in mycotoxicosis includes *Aspergillus flavus,* which produces aflatoxin that causes cancer of the liver, *Aspergillus ochraceous* and *Aspergillus niger* which produces ochratoxin that is nephrotoxic (Ogunledun, 2007). In view of this, there is need to determine the mycological safety of the cocoa powder (CP) we consume as health drink so as to stem down the occurrences of mycotoxin associated diseases in our community.

MATERIALS AND METHODS

Proximate analysis was carried out according to the method of AOAC (2000). Yeast and mould counts were determined using McFaddin (1980) methods. Direct plating of the samples on agar media was carried out by aseptically plating 1g of each sample on potato dextrose agar (PDA). The plates were incubated under room conditions (28 ± 2°C) and examined after 7 days under a stereoscopic binocular microscope for the presence of fungi.

Colonies of fungi that appeared on agar plates were repeatedly sub cultured on fresh PDA until pure culture of each isolate was established. Identification of fungi was done by observing the growth features of the fungi on plates and their morphological characteristics under a binocular microscope. Pure culture of the fungal isolates was placed on clean glass slide on which 2 - 3 drops of lactophenol cotton blue has been added. The fungal mycelia were teased out with the aid of a mounting needle and their hyphae/asexual structures were viewed under compound microscope and identified with reference to standard texts (Barnett and Hunter, 1987). Observation was done under × 40 objective. Characterisation of the fungi was done using War cup (1957) method. It was based on the colour of their colony, appearance, conidiophore, mycelium, arrangement of conida on sterigmata. Data analysis was done with statistical package for social sciences (SPSS) version 15. All quantitative data were analysed by student's t-test and Analysis of Variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to separate the means of the experimental data. Occurrences of mycoflora in the cocoa samples were expressed in frequency and simple proportions.

RESULTS AND DISCUSSION

Table 1 depicts the inherent properties of the various brands of cocoa powder based on the nutritional composition which includes percent moisture, fat, protein,

Table 2. Cocoa powder brand with fungal isolates.

S/N	CP BRAND	Moisture	Mean pH	Fungal isolates
1	CIL	1.1	6.4	*Aspergillus melleus*
2	FRL	1.4	6.4	*Penicillin chrysogenum*
3	IOCP	0.9	6.9	Nil
4	MNL	1.2	6.6	*Aspergillus niger*
5	ONP	1.3	6.9	Nil
6	SCP	1.2	6.9	Nil
7	SF	0.6	7.0	Nil
8	FCP	0.8	6.9	Nil
9	PNL	1.5	6.8	Nil
10	MTIL	1.6	6.4	Nil
11	NNP	0.6	6.6	Nil
12	CNL	0.9	6.4	Nil
13	NBC	1.2	6.5	*Aspegillus ochraceous*
14	EFCO	0.7	6.9	*Penicillium chrysogenum*
15	CCP	0.8	7.0	Nil
16	BAAK	1.3	7.1	*Saccromyces cereviciae*
17	SUMAL	1.2	7.0	*Saccromyces cereviciae*
18	CRIN	0.7	6.9	Nil
19	LI	1.9	7.0	*Saccromyces cereviciae*
20	L2	1.4	6.6	*Aspergillus niger*
21	L3	1.8	6.6	*Aspergillus niger*
22	L4	1.3	6.5	*Aspergillus ochraceus*
23	L5	0.9	6.4	*Aspergillus ochraceus*
24	GCP	0.6	7.0	Nil

ash, carbohydrate contents and their respective energy values. On comparison of mean content values from the various brands of cocoa samples, significant difference was observed in all the parameters except for moisture content. The cocoa powder is nutritious enough to support the growth of microbes when the conditions are favourable. The proximate analysis of the samples of cocoa powder as reported in Table 1, showed variation in the percentage moisture content from 0.80 to 2.86%. These however, falls below the maximum limit of 5% specified for cocoa powder. There are lots of variation in the percentage fat, protein and carbohydrate as well as the pH values of the different cocoa powder samples. The variation that resulted from the final composition of cocoa powder depend on the different roasting temperature, method employed in extracting cocoa butter and alkali treatment employed on the cocoa samples. The fat content of the samples of cocoa powder ranges from 10.20 to 12.72. Fat is important as it contributes to the overall energy value of foods. The variation in fat composition may be due to the different hydraulic method of processing employed by individual companies for the extraction of cocoa butter thereby, varying the percentage of fat that is left in the final cocoa powder. Protein content varied from 18.92 to 22.54%; and carbohydrate is between 54.55 to 60.07%.

Table 2 showed the different cocoa powder brand in relation to the moisture and pH with their respective fungal isolate. This table showed that *A. niger* is the most prominent organism. The pH values ranged from 4.9 to 8.5 and the percentage moisture varies between 0.6 to 1.9%. There is a lot of variation in the pH of the cocoa powder samples varying from 4.9 to 8.5. These wide gaps can be due to variation in the alkaline treatment the cocoa powders were subjected to in the different processing plants. The low pH values are indication of natural cocoa powder while the high values could be attributed to extreme treatment with sodium and potassium carbonate. The pH values fall within the range at which toxigenic mould can grow. The observed pH of cocoa powder in this study corroborated the reports of Wheeler et al. (1991) who further linked pH range above 2.0 – 11.2 as being influential to the growth of some toxigenic species of Aspergillus, Penicillum and Fusarium. The percentage mean moisture varies between 0.6 to 1.9%. This moisture contents were below the NAFDAC recommended maximum allowable moisture content of 5% in cocoa powder. Cocoa powder is hygroscopic, as high moisture will completely reduce the shelf life of the cocoa powder. There is a relationship between moisture content, nutritional composition, temperature and microbial growth. At any temperature, the ability of

Table 3. Occurrence of mycoflora in cocoa powder.

Cultural and microscopic features	Possible isolates	N	n	%
Blackish-brown often with yellow mycelium. Reverse greenish-yellow to yellow-or Its head globose, splitting with age. Its metulae is long, closely packed and brownish.	*Aspergillus niger*	12	3	(25)
White to yellow mycelium. Yellow, buff to brown sclerota. Pale yellow /gold or cream coloured conidia. Uncoloured exudates when present.	*Aspergillus melleus*	12	1	(8.3)
Yellow-buff coloured colonies, small and nearly smooth conidia, pink to purple sclerotia. Uncoloured, yellow or dull red exudates when Present.	*Aspergillus ochraceus*	12	3	(2.5) nearly smooth conidia, pink to purple sclerotia.
The texture is sulicete and velvetious. Bluish-green to (dark) green observed. Its reverse is yellow (occasionally creamish). It has a short smooth strupe. The penicillin is terverni-culete, phialides ampulli form, collula very short, both Divergent and appressed branched. The conidia is allipsoidal to spherical, smooth and greenish.	*Penicillium chrysogenum*	12	2	(16.7)
It is creamish in colour, obverse and oval in shape (spore). The cellular is smooth and very small. It has branched cells (spores)	*Saccharomyces cerevisae*	12	3	(25)

micro-organism to grow is reduced at low moisture content over which growth occurs.

The cultural and microscopic features of fungal isolates were shown in Table 3 with *A. niger* and *Saccharomyces cerevisae* having isolation rates of 25% each, *Penicilium chrysogenum* 16.7%, *Aspergillus melleus* 8.3% and *A. ochraceous* 2.5%. The presence of *Aspergillus* spp is of great public health importance. This table showed that *A. niger* is the most prominent organism. The presence and survival of moulds, already reported to be toxigenic, suggests an imminent public health danger since their metabolites (mycotoxins), if produced in foods like cocoa may lead to serious and devastating clinical conditions in the consumers. There are enough scientific evidences to conclude that naturally occurring aflatoxins and ochre-toxins are carcinogenic to animal and humans (IARC, 1993). Some mycotoxins are tremogenic, as they have been reported to cause novel neurotoxic effects and muscular tremors in animals. Tremorgens are produced by species of *Aspergillus and Penicillium*. Also, they have been known to produce mycotoxins such as aflatoxin, ochratoxins, aflativem, aspergillus and aspertoxin (Oyetunji, 2005).

Aspergilli are among the most abundant and widely distributed organisms on earth. Virtually all the common aspergilla have been recovered at same time from agricultural products. The main impact on agriculture is in saprophytic degradation of products before and after harvesting and in production of mycotoxins. Members of the genus *Aspergillus* have been reported to be more heat tolerant and Xerophilic than most other fungal general (Pitt and Hocking, 1997). These unique attributes must have enhanced their survival, despite the drying and roasting processes and also in the presence of high Osmotic pressure in the cocoa powder brands.

The presence of *Aspergillus species* and *P. chrysogenum;* lipolytic and toxigenic moulds (Uraih and Ugbadu, 1980) should be viewed with great concern since in recent years food poisoning outbreak have been traced to contamination of food products by these organisms (Ormy and Norvorming, 1968).

Conclusion

The result of this study has shown that cocoa powder samples are prone to contamination by xerophilic moulds of public health importance which includes *A. niger, A. ochraceus* and *P. chrysogenum.* It was further observed that, the methods of cocoa processing being employed by the various processing plants were insufficient in preventing contamination by xeropilic moulds which have

high tendency to produce mycotoxins.

Further study is therefore required to elucidate the mechanism by which xerophilic and spore bearing fungi survives in cocoa powder samples with the aim of developing an advanced technique to fore-stall or neutralise this.

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