

Full Length Research Paper

# Mycological evaluation of a ground cocoa-based beverage

T.O. Oyetunji

Department of Microbiology, Olabisi Onabanjo University, PMB 2002, Ago-Iwoye, Nigeria.

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Cocoa beans (*Theobroma cacao*) are processed into cocoa beverage through fermentation, drying, roasting and grinding of the seed to powder. The mycological quality of 39 samples of different brand of these cocoa – based beverage referred to as ‘eruku oshodi’ collected from 3 different markets in south – west Nigeria was determined by serial dilution using the pour plate method on Potato Dextrose Agar (PDA) and Harold agar. The most frequent genera of moulds in the samples investigated were *Aspergillus* and *Mucor* with each having an incidence of 43.6%. The other moulds recovered in decreasing order of occurrence were *Rhizopus* (23.1%), *Penicillium* (18.0%) and *Mycelia sterilia* (18.0%). The yeast recovered was mainly *Saccharomyces*. The health implications of the isolated fungi were highlighted thus calling for improved hygienic practices in production of the beverages.

**Key words:** Ground cocoa-based beverages, moulds, yeast.

## INTRODUCTION

Ground cocoa-based beverages are very common food drinks in Nigeria. These cocoa beverages are known to contain essential minerals such as iron, calcium, phosphorus and vitamins (vit. A, B<sub>1</sub>, B<sub>2</sub>, D). The seeds of cocoa (*Theobroma cacao*) is fermented for about 2 – 12 days to remove sugary pulp surrounding it, to convert the bitter astringent taste into a sweet one and to improve their colour and flavour. At 1 – 2 day intervals throughout the fermentation, beans are thoroughly mixed and then transferred to another box and covered again. As a result of yeast and bacteria growth the temperature rises to between 45°C and 50°C during the first week and then declines (Roelofsen, 1958). Yeasts are the dominant group of microorganisms during the first 1 – 2 days of fermentation. Lehrian and Patterson (1983) compiled a list of several genera isolated from cocoa fermentation by various researchers. They include *Candida*, *Debaromyces*, *Pichia*, *Rhodotorula*, *Schizosaccharomyces* and *Trichosporon*, all of which are yeasts. Cocoa is then dried artificially using an oven or naturally to a moisture content of 6–8%. In the manufacture of cocoa beverage, the beans are roasted at 93–140°C (200–280°F for a maximum period of 2 h to reduce the moisture content to 2.5 – 4% (Anon, 1968). Fermentation and drying are particularly important since they are largely responsible for the typical cocoa flavour precursors which develop later during the roasting of the beans and for the keeping quality of the raw beans (Niles,

1981). Broadent and Oyeniran (1968) also observed the growth of filamentous moulds such as *Aspergillus flavus*, *A. niger*, *A. tamarii*, *A. ochraceus* growth inside fermented beans if drying was prolonged or inadequate or if dried beans were accidentally wetted. A few species including *Aspergillus fumigatus* and *Mucor pusillus* can withstand the temperature reached during fermentation.

Cocoa beans are susceptible to spoilage during and after fermentation. *Aspergillus*, *Mucor*, *Penicillium* and *Rhizopus* species develop on the surface of fermenting heaps which have been turned infrequently or not at all. *Aspergillus*, *Mucor*, and *Penicillium* species grow on cured cocoa beans which have been mishandled or improperly dried (Roelofsen, 1958).

Broadent and Oyeniran (1968) isolated nine moulds from fermenting cocoa beans in Nigeria of which certain species particularly *A. flavus* could have produced toxic metabolites which may constitute health hazard to man and animals. Cocoa-based beverage is a reduced water activity (aw) food that may not constitute suitable substrate for the growth of microbes except the xerophilic and osmophilic organisms that are able to survive and grow in this substrate. Moulds differ considerably in optimal aw for germination of the asexual spores. The minimal aw activity for spore to germinate has been found to be as low as 0.62 for some moulds and as high as 0.93 for others such

**Table 1.** Total fungal counts per gram of beverage.

Beverage	Mould count (10 <sup>3</sup> cfu/g)	Yeast count (10 <sup>4</sup> )
Bestvita	1.8	15
Solo drink	1.5	13
New life	1.2	10
Fruit chocolate	0.9	7

as *Mucor* and *Rhizopus* (Frazier and Westhoff, 1978). According to these authors, microbial contamination of commercially processed food often causes food spoilage, food-borne poisonings, infections and intoxication. Some of the ingredients used in processing of ground cocoa-based beverage like egg, cocoa, provide different condition for microbial growth and thus differ in their microbial population (Pelczar et al., 1993). In the past few years, different ground cocoa-based beverages have been introduced into the Nigerian market by various manufacturers bearing different names with attractive labels. Most of these beverages are produced without the involvement of a food microbiologist or scientist to ascertain the quality and safety of products.

Cocoa-based beverages are supposed to be produced and packaged under hygienic condition and they are assumed to be safe for human consumption. However, the newly introduced beverages might be receiving less heat treatment and materials used in formulating them might have been contaminated with aerosols and dust carrying organisms. Therefore, the overall purpose of the research into the mycological evaluation of these products is to have an insight into the mycological quality of the beverage and the possible health hazards that may be posed unto the consumers.

## MATERIALS AND METHODS

### Collection of samples

Thirty-nine (39) samples of different brands of the beverages (Bestvita, Solo drink, New life and Fruit chocolate) were obtained from three different markets (Oshodi, Ijebu – igbo and Ago – Iwoye) in southwestern Nigeria. The samples were obtained at two-week intervals for 10 weeks to obtain a good representation. Samples were analysed mycologically within 24 h of collection.

### Evaluation of mycoflora

The evaluation of fungi was carried out using dilution plating method and the direct plating technique. Decimal dilutions of the samples were carried out by placing one gram (1 g) of the cocoa powder into 9 ml of sterile distilled water. This was thoroughly shaken and from the suspension, 1 ml was transferred to another tube containing 9 ml of sterile distilled water and thoroughly mixed again. This dilution procedure was further repeated thrice so that there were series of five tubes giving a serial dilution of 10<sup>-1</sup> to 10<sup>-5</sup>. An aliquot of 1 ml was pipetted at each dilution into six sterile Petri dishes. Three of the plates were over-laid with cooled molten potato

dextrose agar (PDA). The remaining three were over-laid with Harold agar. The latter contained malt, 40% sucrose and yeast extract which makes it suitable for isolating osmophilic and xerophilic moulds. Each medium was supplemented with 0.60 µg/ml of streptomycin sulphate to suppress the bacterial growth. The plates containing the cocoa powder and melted agar were swirled round to allow for thorough mixing of aliquot and media. After the agar had gelled, the plates were incubated at room temperature (28±2°C) for 5 to 7 days. The number of fungal colonies that appeared in a plate was multiplied by the dilution factor to obtain the number of colony forming units per gram (cfu/g) of cocoa beverage.

For direct plating on agar media, 1 g of each sample was aseptically plated on PDA and Harold agar. 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.

Representative 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 by observing the growth habits and morphological characteristics under a wild binocular microscope. Wet mounts of hyphal/asexual structures stained with lactophenol in cotton blue were viewed under the compound microscope and identified with reference to standard texts (Barnett and Hunter, 1987).

### Estimation of total fungal counts

The total fungal in the samples was estimated from the decimal dilutions carried out. The incidence of mould contamination using direct inoculation was expressed as a percentage of the 39 samples examined.

## RESULTS AND DISCUSSION

The total mould counts in the samples varied from 0.9 x 10<sup>3</sup> to 1.8 x 10<sup>4</sup> cfu/g while the yeast count ranged from 7 x 10<sup>3</sup> to 15 x 10<sup>4</sup> /g (Table 1). Altogether, 12 fungal species belonging to 6 genera were identified. *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* accounted for most of the mould isolated while *Saccharomyces* and *Schizosaccharomyces* were the main yeasts isolated. Their incidence is presented in (Table 2.) Bestvita has the highest count followed by solo drink. Species of genus *Aspergillus* were mainly isolated. It has been demonstrated that most of these food-borne fungi exhibit the potential to produce toxic metabolites. There is sufficient evidence to conclude that naturally occurring mixtures of aflatoxins are carcinogenic to animals and humans (IARC, 1993).

Some mycotoxins are tremorgenic i.e. cause novel neurotoxic effects; muscular tremors in animals. Tremorgens are produced mainly by species of *Aspergillus* and *Penicillium*. Also, they have been known to produce mycotoxins such as aflatoxin, ochratoxins, aflatrem, aspergillilic acid and aspertoxin. Mycotoxins seem able to cause serious disease of the liver, kidney and blood – forming organs in extremely low quantities i.e. parts per billion. In addition, many mycotoxins have been shown to impair immunity against various pathogenic agents. This has been demonstrated for aflatoxins, diacetoxyscripenol, ochratoxin and rubratoxin (Pohland, 1993; Scudamore et

**Table 2.** Incidence of fungi in cocoa beverage samples.

Sample	Total no. of samples	<i>Aspergillus</i> sp.*	<i>A. flavus</i>	<i>A. niger</i>	<i>Aspergillus</i> sp. 1	<i>Aspergillus</i> sp. 2	<i>Aspergillus</i> sp. 3	<i>mycelia sterilia</i>	<i>Mucor</i> sp.	<i>Rhizopus</i> sp.	<i>Penicillium</i> sp.	<i>Saccharomyces</i>	<i>Schizosaccharomyces</i>
Bestvita % incidence	12	5 12.8%	2	5	2	2	1	-	6 15.4%	5 12.8%	3 7.7%	10 25.6%	-
Solo drink % incidence	10	4 10.3%	3	1	2	2	-	6 15.4%	4 10.3%	2 5.1%	-	8 20.5%	-
New life % incidence	10	5 12.8%	1	4	1	3	2	-	5 12.8%	2 5.1%	2 5.1%	7 17.9%	2 5.1%
Fruit chocolate % incidence	7	3 7.3%	-	2	-	3	1	1 2.6%	2 5.1%	-	2 5.1%	5 12.8%	-
Overall total	39	17 43.6%						7 18.0%	17 43.6%	9 23.1%	7 18.0%	30 76.9%	2 5.1%

\*The different species of *Aspergillus* and number of samples contaminated are further specified in following columns.

al., 1993). Again, in humans, mycotoxins have been implicated in a form of encephalopathy observed in Thailand and in a particular nephropathy rather frequently seen in the Balkans (Betina, 1989).

Apart from danger of food poisoning caused by these fungi, they also utilize nutrients found in the food thereby causing deterioration of such food. To improve the quality of cocoa beverages and to prevent spoilage at various  $a_w$ , it was suggested by Mossel and Shennan (1976) that if the  $a_w$  is below 0.65 and the product is maintained at this level during storage, problems arising due to microbial spoilage are rare, irrespective of the number of contaminating organisms present. It was further noted that aflatoxins

production ceases or become very low at  $a_w$  below 0.85 (Diener and Davis, 1969). However, high level of contaminating organisms should be avoided, since they can survive for long periods and may contaminate other foods or cause problems after dehydration.

Pelczar *et al.*, (1993) also reported that the extent of contamination will depend upon the initial microbiological quality of the product and the level of aseptic precaution used during handling. Therefore, if the product is well handled, the level of microbial content of the final product will be minimal.

Furthermore, the spectrum of the fungi isolated is similar to those in the raw material. This may be due to re-infection of the product during cooling of the samples before they are packaged into polyethylene bags because of the ubiquity of these organisms. A more recent and

increasingly popular way of preserving foods is the use of controlled storage (CA) or modified atmosphere packaging (MAP). These methods take advantage of combining the inhibitory effect of low  $O_2$  levels and elevated  $CO_2$  levels in any deterioration processes in foods as well as preventing the microbial spoilage (Haasum and Nielsen, 1998).

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