Technical Report

Microbial contaminants of commercially bottled non-alcoholic drinks produced in Nigeria

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When microbiological analyses were conducted on 90 samples of soft drinks representing 30 different products commercially available in Nigeria, contaminants were detected in 59% of them. The isolates were mainly saprophytic and non-pathogenic: *Bacillus* spp. (35%), *Lactobacillus* spp. (26%), *Pediococcus* spp. (6%), *Staphylococcus epidermidis* (6%) and *Mucorococcus* spp. (3%) accounted for the bacterial isolates while *Aspergillus niger* (6%) and *Sacharomyces* spp. (16%) accounted for the fungal isolates.

Key words: Bacteria, microbial load, moulds, non-alcoholic drinks, yeast.

Non-alcoholic beverages are highly prone to microbial contamination (Odunfa 1987). While high-level microbial contamination can cause economic loss through product spoilage and consumer rejection, lower and usually inconspicuous levels may, if uncontrolled, pose grave human health problems (Frazier & Westhoff 1978).

The recent economic hardship in Nigeria has led to an outright ban on the import of the raw materials traditionally used for the production of various beverages, including non-alcoholic. This has led to the use of local and unconventional raw materials for the purpose (Ogbonna & Obi 1991) and to changes in production practice, product quality and shelf-life. The present report is on the microbiological profile of the entire spectrum of the bottled non-alcoholic beverages now available in Nigeria.

Materials and Methods

**Sampling Method**

Non-alcoholic beverages (three bottles each) were purchased from retail outlets across Nigeria. The bottles were cleaned externally with absolute ethanol to disintect and sampled using two distinct sampling methods.

**Method I.** From each alcohol-cleaned bottle, 100 ml were removed and filtered either through a 0.4-μm pore membrane-filter for bacteria or a 0.8-μm pore filter for yeasts and moulds (Odunfa 1987). The membranes were then placed on appropriate nutrient media and incubated at 30°C for 48 h.

**Method II.** Triplicate aliquots of beverage samples (100 ml each) were placed in sterile centrifuge tubes, centrifuged at 15,000 x g for 30 min, and supernatants decanted aseptically, leaving about 0.5 ml in each tube. After vigorous agglutination, these residues were used to inoculate appropriate nutrient media by spreading on agar plates and were then incubated at 30°C for 48 h.

**Identification of Isolates**

Bacterial colonies from Methods I and II were streaked on standard plate count agar, tomato juice agar or MacConkey agar (all Oxoid) plates while yeasts and moulds were inoculated on Sabouraud's dextrose agar. The bacteria were identified microscopically and by biochemical and physiological tests, with reference to standard identification manuals (Buchanan & Gibbons 1970). The yeasts and moulds were identified by microscopy and sugar assimilation and oxidation tests (Koering van Rij 1984).

**Statistical Analyses**

All statistical analyses were by Student's t-tests.

**Results**

Mean c.f.u. values of 19.7 and 31.0/100 ml sample were recorded using Methods I and II, respectively. Statistical analyses of the rates of microbial isolation showed that Method II gave significantly more isolates (P = 0.001) than Method I. The different microbial contaminants isolated from the non-alcoholic beverages (listed in Table 1) included bacterial mould and yeast species. The order of occurrence of specific bacterial...
ages in Table 1) included bacterial, mould and yeast species. The order of occurrence of specific bacterial genera was as follows: *Bacillus* > *Lactobacillus* > *Pediococcus* > *Staphylococcus* > *Micrococcus*. Bacteria accounted for 72% of the total microbial isolations while fungal isolates (Saucesohernospora spp. and *Aspergillus niger*) accounted for the remaining 23%. The rates of isolation of the different bacterial and fungal genera from individual non-alcoholic beverage groups are shown in Table 2. The trend for microbial isolation was as follows: soda drinks > cola drinks > orange drinks > lemon/miscellaneous drinks = malt drinks.

**Discussion**

Most of the isolates from the non-alcoholic drinks were saprophytic. Except for *Staphylococcus epidermidis*, which is sometimes an opportunistic pathogen (Buchanan & Gibbons 1974), no pathogen was isolated. The non-malt drink categories (i.e. cola, orange, soda, and lemon/miscellaneous) together accounted for > 50% of all the microbial isolations, with the soda group having the highest value of 32% (Table 2). This situation may not be unconnected with the pH values of the soda group (Table 2), which were generally > 3.5 and therefore judged microbiologically unsafe for this category of products (Frazier & Westhoff 1973). The malt drink category presented one of the lowest rates of microbial isolation (10%) and this may be attributed to the rigorous process of malt drink preparation, which includes hop addition and boiling, together with final pasteurization. This ensures that any microbial contaminants introduced at the various earlier stages of production are eliminated. The generous components of hops are known to possess broad spectrum anti-microbial potency. The malt drinks examined gave bitterness values, due to hops, of between 12 and 14 E.B.U.

Critical study of the isolates shows that the non- and thermo-tolerant *Bacillus* spp. (Frazier & Westhoff 1973) predominated. These organisms, which are also salt-tolerant and produce thermo-resistant spores, can survive preservative concentrations much higher than those normally used in non-alcoholic beverages (Olufade 1987). More importantly, being environmentally occurring, they may have been introduced into these drinks from either the environment and/or the raw materials (Frazier & Westhoff 1978). The *Lactobacillus* spp. were the next major group of isolates. Their widespread occurrence in soft drinks has been widely established, being associ-
ated with their 'special adaptative properties' (Otunuga 1987). As common food contaminants, they are very acid-, CO₂-, and salt-tolerant, and can grow well in the presence of 30% (w/v) sugar (Frazier & Westhoff 1970). Other common contaminants of food and beverages that were isolated include: Pediococcus spp., Aspergillus niger and Saccharomyces cerevisiae.

Their isolation is in agreement with earlier reports linking them with beverage and soft-drink factory environments (Otunuga 1987). Staphylococcus epidermidis and Micrococcus species are anaerobic bacterial members of the normal human flora (Bushman & Gibbons 1974). Their presence in the non-alcoholic drinks may have been the result of contamination from factory personnel.

References


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