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Microbial Profiling of Coca-Cola and Fanta Orange Using the Hazard Analysis Critical Control Point (HACCP) Concept in a Beverage Production Plant

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ABSTRACT

Investigation of Coca-Cola products (Coke and Fanta Orange) involving (the raw materials, simple syrup, final syrup and final products) was carried out using the hazard analysis critical control point concept. The production environment was also monitored for microbial quality. The total viable counts (TVCs) of the water samples varied from raw water to water obtained from polishing filter with the highest (7.5 x l0^2 cfu ml^{-1}) occurring in the former. Coliform counts of the water samples also showed variations ranging from 1.0 x 10^2 cfu $m1^{-1}$ to 5.0 x 10^2 $cfuml^{-1}$ The total viable counts (TVCs) of the sugar samples (2.0 x 10^2 $cfug^{-1}$) while the fungal counts 1.5 x 10^2 $cfumg^{-1}$. The total viable counts (TVCs) of simple syrup ranged from 5.0 x10^2 cfum-^{2} to 7.0 x 10^2 $cfuml^{-1}$ while the fungal loads ranged from 5.0 x 10^2 $cfuml^{-1}$' to 8.0 x 10^2 $cfum^{-l}$. The total viable counts (TVCs) of final syrup were different for Coke and Fanta Orange, with the higher counts of 2.0 x 10^2 $cfum^{-1}$ occurring in Fanta Orange while the fungal counts were 4.0 x 10^2 $cfuml^{-1}$'. The total viable counts of the final products (Coke and Fanta Orange) differed on the day of production with the former (Coke) showed higher populations of 5.0 x 10^2 $cfuml^{-1}$. The total viable counts and fungal loads were higher in Fanta than in Coke ten days of ambient storage after production. The production environment (simple syrup room, final syrup room, washer II outlet and filler line II area) showed variations in microbial profiles with filler line II showing the maximum (14.0 x 10^2 $cfum^3$ for fungal counts, 7.0 x 10^2 $cfum^{-3}$ for coliform and 25.0 x 10^2 cfum-^{3} for total viable counts respectively) and the minimum in simple syrup room 1.1 x 10^2 $cfum^3$ for fungi; 5.0 x 10^2 $cfum^{-3}$ for coliform and 1.0 x 10^2 $cfum^3$ for total viable counts respectively. The carbonation level of the product (Coke! Fanta) differed also with Coke having 3.80 and Fanta 2.80. The isolated samples were identified as *Bacillus spp, Leuconotsoc spp* and *Lactobacillus sp*p with Bacilis spp being more predominant in the environment. The fungi were identified as *Fusarium spp, Penicilliurn spp, Aspergillus spp* and *Geotrichum spp* with *Fusariurn spp* being more predominant. The pH of raw water (Borehole water) to final product (Coke and Fanta) ranged from 5.00 borehole water, 6.60 sand filtered water, 6.80 carbon filtered water, 4.00 Coke and 4.11 for Fanta Orange respectively. This work has shown that the microbial characteristics of the final product (Coke and Fanta Orange) are influenced by the quality of the raw materials and the measures employed in the production process.

INTRODUCTION

Non-alcoholic beverages include carbonated and non-carbonated non-alcoholic soft drinks. The major sources of the raw materials include plant parts such as cereals, cocoa beans, soyabeans, fruits and concentrates. Non-alcoholic beverages have a variety of names such as fruit drinks and juices. Irrespective of the name, they are generally mixtures of water with fruit juice, sugar, various colourings or flavourings or pulverized extracts or leaves. The production processes differ from beverage to beverage. Nonalcoholic beverages have always been of interest globally, due to their importance in global economy and international trade. There is an increasing demand for food safety world wide. The those significant driving force for increased attention in food safety has been the continued surge in emerging food borne out-breaks. Carbonated beverages are consumed by children, teenagers and adults for many reasons including refreshment, relaxation, pleasure and simply for thirst-quenching capacity. “Soft drinks” in this work are referred to as carbonated non-alcoholic drinks made from sugar syrups and fruit flavoured concentrates that are appropriately diluted.

The Nigerian Bottling Company (NBC) is one of the authorized bottlers of Coca cola products in Nigeria. These products include Coke, Vanilla Coke, Fanta apple, Fanta lemon, Fanta orange, and Schweppes soda. The products, Coke and Fanta are the most popular worldwide (Hollingsworth, 1997). Quality control is the cumulative effects of all controllable factors that influence positively the quality of finished products. This assures that the finished beverage is produced at the lowest possible cost and its quality image maintained and very competitive in the market until purchased and consumed.

Microbial contamination of Soft drinks can cause economic loss through product spoilage and consumer rejection. Sources of contamination of carbonated beverages may include: (i) Raw materials (water, sugar), (ii) Factory personnel especially those that harbour Staphylococcus epidermidis, and (iii) Factory environment especially spore formers and moulds. This implies that uncontrolled manufacturing services can pose grave human health hazards (Higgins, 2002). The

distribution practices of food and beverages is as important variable as the production processes, hence they constitute part of the Hazard Analysis Critical Control Point (HACCP) concept. Similarly, the need for the storage tests or shelf-life studies to provide information on expected microbial changes following distribution until consumption has been highlighted (Notermans and Mead, 1996).

Storage of food at low temperature is encouraged based on retardation of enzymatic activity of microorganisms. As temperature decreases, the enzymatic activity of microorganisms also decreases and eventually stop at below. Psychotrophic bacteria and moulds can spoil food under prolonged low temperature storage. Although most psychotroph are non-pathogenic, some pathogens such as Clostridium botulinum and Listeria monocytogenes have been reported to grow at 4-6°C (Tompkin, 2002). On the other hand, storage of food and beverages at high temperatures enchance microbial growth and food spoilage. Several complaints of microbial spoilage and questionable safety of soft drinks re frequent in Nigeria and they are of public health concern. Therefore, the objectives of the present work were to:

(1) Employ the Hazard Analysis Critical Control Point (HACCP) concept with emphasis on the microorganisms associated with the raw materials (raw water, sugar, simple syrup, and fmal syrup)
(2) Analyse samples at critical points for microbial contaminants during the production process
(3) Determine the microbial quality of the fmal product and
(4) Evaluate the pH values of the selected samples.

Origin of Coca- Cola products

Coca-cola is a cola (a type of carbonated soft drink) sold in stores, restaurants and vending machines in more than 200 countries (Codex Alimenterius Commission I (1997)). It is produced by the Coca-cola company and is often referred to simply as coke. It was originally intended as a patent medicine when it was invented in the late 19th century by John Pemberton. Coca-cola was brought about by business man Asa Griggs Candler, whose marketing tactics resulted to its dominance of the world soft drink market throughout the 20th century. Although faced with criticisms of its health effects and various allegations of wrongdoing by the company, Coca-cola has remained a popular soft drink to the present day. The company actually produces concentrate for Coca-cola which is then sold to various licensed Coca-cola bottlers throughout the world. The bottlers who hold territorially exclusive contracts with the company, produce finished products in cans and bottles from the concentrate in combination with filtered water and sweetners. The bottlers then sell, distribute and merchandise Coca- cola in cans, and bottles to retail stores and vending machines. Such bottlers include Coca-cola Enterprises which is the single largest Coca-cola bottler in North America, Australia, Asia and Europe the Coca-Cola Company has, on occasion, introduced other Cola drinks and under the coke brand name, the most common of these is Diet coke which has become a major diet cola. Others include diet coke, caffeine free, cherry coke, vanilla Coke and Fanta orange. the first coca-cola recipe was invented in Covington, Georgia, by John Stith Pemberton, originally as a coca wine called Pemberton's French wine coca in 1885 (IL.S, Europe (2004).

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Coca-Cola and its Composition

In 1885, when Atlanta and Fulton Country (United States) passed prohibition legislation, Pemberton responded by developing Coca-cola, essentially a carbonated, alcoholic version of French Wine Cola. The beverage was named Coca-cola because, originally, the stimulant mixed in the beverage was Coca leaves from South America, from which the drug cocaine is derived. In addition, the drink was flavoured using kola nuts, also acting as the beverage source of caffeine. Coca-cola did once contain an estimated nine milligrams of cocaine per glass, but in 1903 cocaine was removed. After 1904, coca-cola started using, instead of fresh leaves; "spent" leaves (the leftover of the cocaine extraction process with cocaine trace levels left over at a molecular level). To this day, coca-cola uses as an ingredient, a non-narcotic coca leaf extract prepared at a Stepan company plant in May wood, New Jersey. In the United States, Stepan Company is the only manufacturing plant authorized by the Federal Government to import and process the coca plant. Pemberton claimed coca-cola cured many diseases, including morphine addiction, dyspepsia, neurasthenia, headache and impotence. Pemberton ran the first advertisement for the beverage on May 29th 1885 in the Atlanta Journal. By 1888, three versions of coca-cola sold by three separate businesses were on the market.

Canning of Coca-Cola Products

Coca-cola was sold in bottles for the first time on March 12, 1894. Cans of coke first appeared in 1955. The first bottling of coca-cola occurred in Vicksburg, Mississippi at the Biedenham Candy Company in 1891. Its proprietor was Joseph A. Biedenharn. The original bottles were Biedenham bottles very different from the later hobble-shirt design that is now so familiar. The "hobble skirt" bottle was in 1915 by bottle designer, Earl R. Dean. In 1915, the Coca-Cola company launched a competition among its bottle suppliers to create a new bottle for the age that would distinguish it from other beverage bottles "a bottle which a person could recognize even if they felt it in the dark and so shaped that, even if broken a "contour can", similar in shape to their famous bottle, on a few test markets including Terre Haute, Indiana. This new can was however never widely released. In 2007, coca-cola Canada changed "coca-cola the "classic" designation, leaving only "coca-cola". Coca-cola stated

this is merely a name change and the product remains the same coca-cola is a registered trade mark in most countries around the world and should always be written with the hyphen and not as “coca cola”. The coca-cola company has been criticized for its business practices as well as the alleged adverse health effects of its flagship product a common criticism of coke based on its allegedly toxic acidity levels has been found to be baseless by researchers. Lawsuits based on these criticisms have been dismissed by several American courts for this reason under normal conditions, scientific evidence indicates coca-cola’s acidity causes no immediate harm. The coca-cola company has responded that its plants filter water to remove potential contaminants and that its products are tested for pesticides and must meet minimum health standards before they are distributed.

Distribution Survey

A filed survey of soft drinks manufacturing and marketing has shown that by June 1982, soft drink factories were located in at least twelve out of nineteen states including Rivers State with some having more than one soft drink factory.

Hazard Analysis Critical Control Point (HACCP) Concept

The hazard analysis critical control point (HACCP) concept is a systematic approach designed to identify and control food -safety hazards (i.e. harmful microorganisms or chemical and physical contaminants) and monitor the controls d. This concept permits a systematic approach to the identification of hazards and an assessment of likelihood of their occurrence during the manufacture, contribution and use of a food product, and defines measures for their control Hazard Analysis Critical Control Point (HACCP) concept was developed in 1959 by the Pisbury Company to supply safe food to NASA (the U.S National Aeronautics and Agency) for its space programmes. The benefits of the system were tremendous and two years after first moon landing the method was introduced to the commercial manufacturing of consumer foods and was applied successfully to the safety control of low acid canned foods. The term Hazard Analysis Critical Control Point (HACCP) was first introduced to the public in America at the 1971 National Conference on Food Protection, with the first comprehensive document being published by the Pillsbury Company in 1973. Numerous food companies have utilized HACCP concepts to evaluate food safety controls and systematically design intervention into their processes. Each food producing facility was encouraged to develop a system of control based on the HACCP principles. These controls should be specific to the facility’s ingredients, processes, equipment, storage and distribution conditions and the intended use and consumer of the product. It was decided that the best way to ensure food safety was to prevent hazards/contamination from occurring during the production process. Since then, Pillsbury’s HACCP system has gained worldwide recognition as a state of-the - art method for food —safety control. The first Food and Drug Administration (FDA) required HACCP controls were for canned foods (1973) to prevent Clostridium botulinum the cause of botulism. In 1985, the National Academy of Sciences recommended that the HACCP approach be adopted by all regulatory agencies and that it be mandatory for food processors. HACCP has been approved by international organizations such as the Codex Alimentarius (a United Nations Commission) and the European Union. Codex Alimentarius Commission (1997). The FIACCP system is not risk free. However, it was developed to minimize the risk of food-safety hazards. HACCP offers an advantage over traditional inspection systems. Traditional methods study the processing practices in use for the day or period inspected, a snap-shot technique. In Contrast, the HACCP approach allows regulators to examine a company’s record about monitoring and corrective actions over a long period of time. A properly completed and implemented HACCP study identifies and controls the factors directly affecting the safety of a product. This allows the food producer to target technical resources efficiently. A food safety objective (FSO) is a statement of the maximum frequency and! or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection. Codex Alimentarius Commission (1997).

Production and Safety of Soft Drinks

The rapid changes in technology with more sophisticated advances being introduced on regular basis led to a greater awareness concerning food safety. This means that manufacturers have to be more knowledgeable about quality parameters. Food safety must take into account all potential hazards emanating from raw materials right through distribution to the consumer.

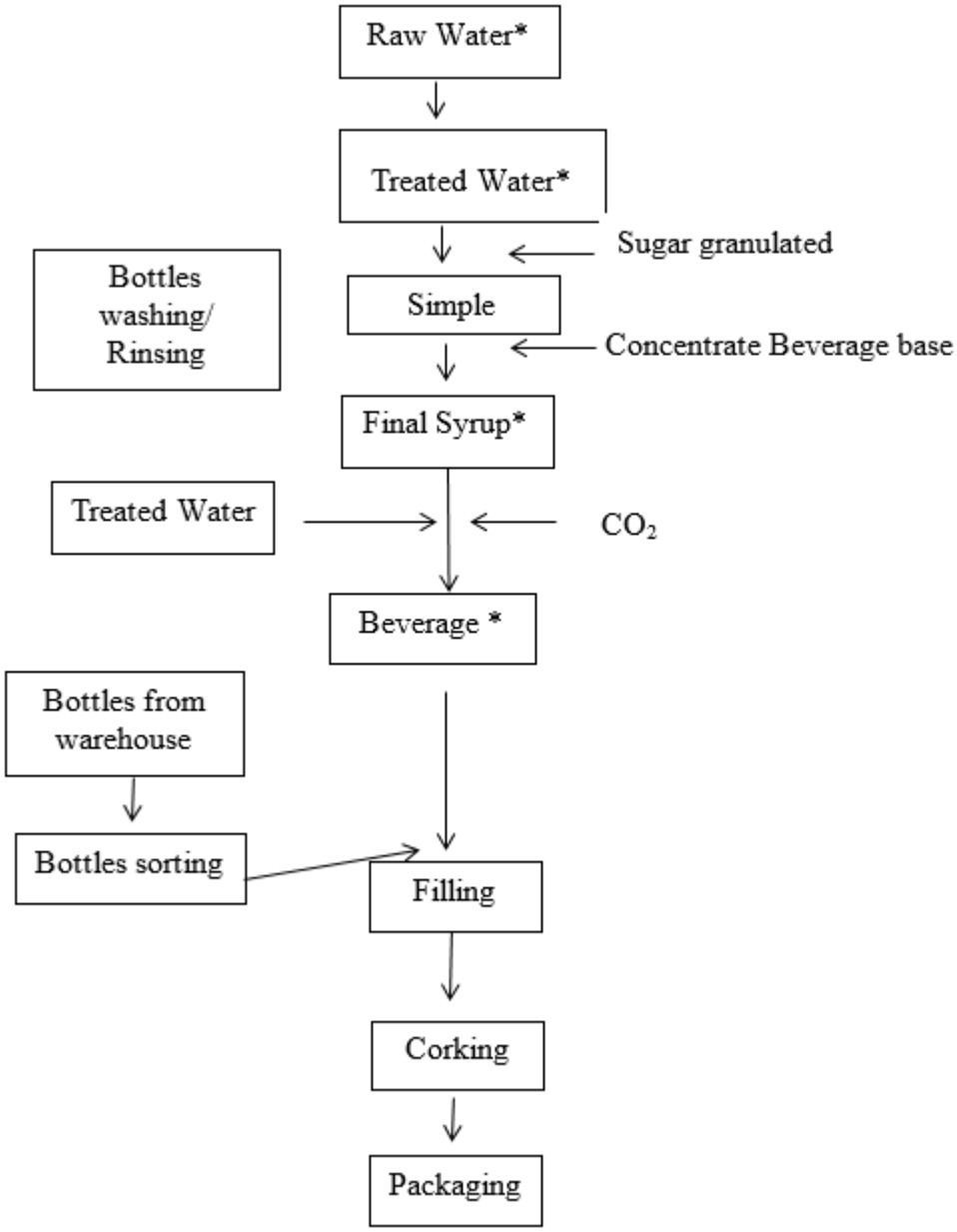


Figure 1: Sampling at Critical Control Point

Raw materials used for the production of Coca-cola include treated water, sugar (granulated sucrose), concentrates and carbonation. Raw water is normally abstracted from boreholes and transferred into raw water reservoir from there into the reaction tanks where chemicals (caustic soda and chlorine) are continuously dosed into it. The water is treated before usage. Granulated sugar (sucrose) was mixed with a known volume of the treated water to form simple syrup. To the simple syrup was added the beverage based concentrate to form final syrup. The beverage or final product is a mixture of final syrup, treated water, and carbon dioxide (carbonation). More treated water was added to the final syrup under carbonation of specific volumes. The final beverage (Coke and Fanta) was packages into clean washed returnable bottles then coded with dates and batch numbers and packaged into crates of twenty-four bottles and then into pallets of eight crates for storage and distribution. The shelf-life of any coca- cola product is guaranteed for twelve (12) months, after which consumption of the product is harmful to the health of the consumer The manufacturing processes for all foods and beverages have to be planned in advance with a clear idea of all components needed. These include raw materials, processing equipment and the human resources needed. As the finished products emerge, certain analysis are carried out to determine the quality of the products, the equipment should be cleaned

and sometimes sterilized (Higgins, 2002). The effectiveness of sanitizing procedures, microbiological testing programmes are carried out. These are to prevent product losses by identifying those areas where cleaning efforts should be concentrated. These tests are carried out on a periodic basis, normally weekly to show a trend. The key ting parameters are organoleptic, visual appearance, tests, odour and microbiological quality as well as chemical parameters. The critical control points include the water treatment system, ingredient quality, finished syrup and products are also given adequate attention.

Microorganisms Associated With Coca-Cola Products and Other Beverages

Many genera of microorganisms including bacteria, yeasts and moulds occur in the raw materials that are used for the manufacturing of soft drinks (Codex Alimentarius Commission, 1997). The types of yeasts present that produce spores (spores formers or non-spore formers may vary depending on the type of fruit concentrates used. For example, citrus fruits concentrates are contaminated mainly with Candida, Saccharomyces and Pichia species. While moulds that occur frequently are caused by *Penicillium species, Cladosporium species*

Byssochiamys_species. However, moulds and yeasts generally have high acid tolerance and are capable of growing in high concentrations of sugar and these are called osmophilia organisms that are high sensitive to salt (Koppenstei0ner and Windisch, 1971) among the bacteria, only the lactic and acetic acid organisms can grow at low pH The growth of lactic acid bacteria causes opalescence of soft drinks and concentrates and sometimes visible bubbles of gas or increased press and damage to containers (Codex Alimentarius Commission, 1997). In general, soft drinks are low risk foods or beverages since growth of pathogens does not take place primarily because of low pH though food borne diseases from these products occur occasionally probably after spoilage organisms have neutralized the natural acids.

MATERIALS AND METHODS

Materials and their sources

The production of coca-cola products include raw material (sugar, water, concentrates, carbonation (C02), crown corks, and returnable glass bottles (RGB). Those analysed were sugar, water, and final beverage.

The sugar was purchased from Dangote Nigeria Limited, the water was obtained from boreholes sank in the company premises which is treated by passing through the sand filter, carbon filter and polishing filters before being used as potable water. The bottles and corks were purchased from Beta Glass Company, Ughelli, Delta State. The concentrates were supplied to Coca-Cola Company from the parent company in Vienna, United States of America.

Location of Plant

The location of the plant in which this work was carried out is the Nigeria Bottling Company Coca-Cola Trans-Amadi, Port Harcourt.

Culture media used

The culture media used in this study were yeast extract agar for determination of total viable counts, Mannitol salt agar for the determination Staphylococci, Potato dextrose agar was used for the enumeration of fungal load, Macconkey agar was used the enumeration of coliform counts (Report, 1999), Nutrient agar was also used to culture isolates for pure culture and identification of the organisms.

Handing and Washing of Bottles

Empty bottles in crates were de-palletized (i.e taken out of the pallets and placed on the roller /conveyor) which carried them to where they were uncased. These ty bottles were inspected prior to their washing. The purpose was to remove bottles with chip-necks, foreign particles or stains. Thereafter, they were sorted and loaded into the washer (bottle washing equipment - krones a returnable glass washer made in Germany). The bottle washing process ensured cleanliness and commercial sterility.

Water Treatment

The raw water abstracted from the boreholes was transferred into raw water reservoir from where it was pumped into the tanks. Chemicals (caustic soda and chlorine) are continuously dosed into the reaction tanks through the flash mixer. The water was then filtered with the aid of sand filters in the buffer tank. The stored in the intermediate storage tanks. This was further purified by passing it through a carbon filter (stainless steel pipe) and further filtered by polishing filters (stainless steel container). Raw water was aseptically collected from the boreholes into sterile containers (100 ml WHIRL-PAK sterile bags), by flaming the mouth of the tap with 75% ethanol. Water samples from raw water to treated water were analysed. Water samples were taken from the boreholes, sand filter I, sand filter II, Carbon Filter I, carbon filter II, Polishing filter I and polishing filter II respectively. 0.1 ml of the water samples was inoculated into solid media of MacConkey Agar and Yeast Extract Agar in duplicate using the spread plate method to enumerate for coliform and TVC (total bacterial count) respectively. The plates were then incubated at 35°C for 24 hours after which colonies that developed were counted.

Syrup Manufacturing / Blending

The treated water was then sent to the flow- mix for mixing with sugar and in the production of the desired products. As part of the production process, the simple syrup was prepared by weighing a known quantity of sugar (sucrose) and dissolving it in a known volume of treated water. It was then filtered and c. The final syrup was prepared by adding concentrates to the filtered simple syrup was homogeneously mixed to give the desired end-product such as Coke and Fanta and then sent to the flow-mix for bottling. 10 gramms of granulated sugar was dissolved in 90 ml of distilled water. A ten fold serial dilution of the simple syrup, final syrup and beverage was prepared. To a set of nine (9) sterile test tubes, 9m 1 of 0.1% peptone water (diluent) was transferred into each test tube using a sterile pipette. A ten fold serial dilution of the simple syrup was prepared by aseptically pipetting Iml of the simple syrup using a sterile lml pipette and transferring to the first tube. This first dilution tube was labeled 10.1. from this, further serial dilutions were prepared up to i0. aliquots of each dilution (10^{1-10}) were aseptically pipetted and inoculated onto solid media of potato Dextrose agar in duplicate using the spread plate method. The plates were incubated in a UNISCOPE laboratory incubator and MEMMERT incubator respectively. The UNISCOPE laboratory incubator was used for total bacterial count and coliform at 35 ± 2°C for 2 to 5 days while the MEMMERT incubator was used for yeasts and mould at 25 ± 2°C for 2— 5 days as well, after which the colonies which developed were counted. This process involved five (5) stages namely;

Inspection of bottles: - After filling the bottles with the beverage already prepared, the bottles were inspected before being coded.

Data Coding: - Each bottle was labeled (coded) before being sent to the filler. This allowed easy identification of where and when it was produced (i.e Batch).

Filling Inspection: - Once the bottles got into the filler, they were filled with the beverage and crowned with corks.

Full Inspection: - After filling and corking, the bottled beverages were inspected once again to ensure that only properly filled bottles free from foreign particles wee allowed passage to the casing table where they were palletized (i.e the cases placed on pallets). At this final inspection point, defective products (improperly filled bottles, over filled or un-crowned bottles) were removed. Casing; at this point the filled and crowned bottles were packaged into the 24-bottle plastic crates. After palletizing, the crates were then carried away by forklifts to the warehouse where they are stored at ambient temperature.

Quality Control Checks

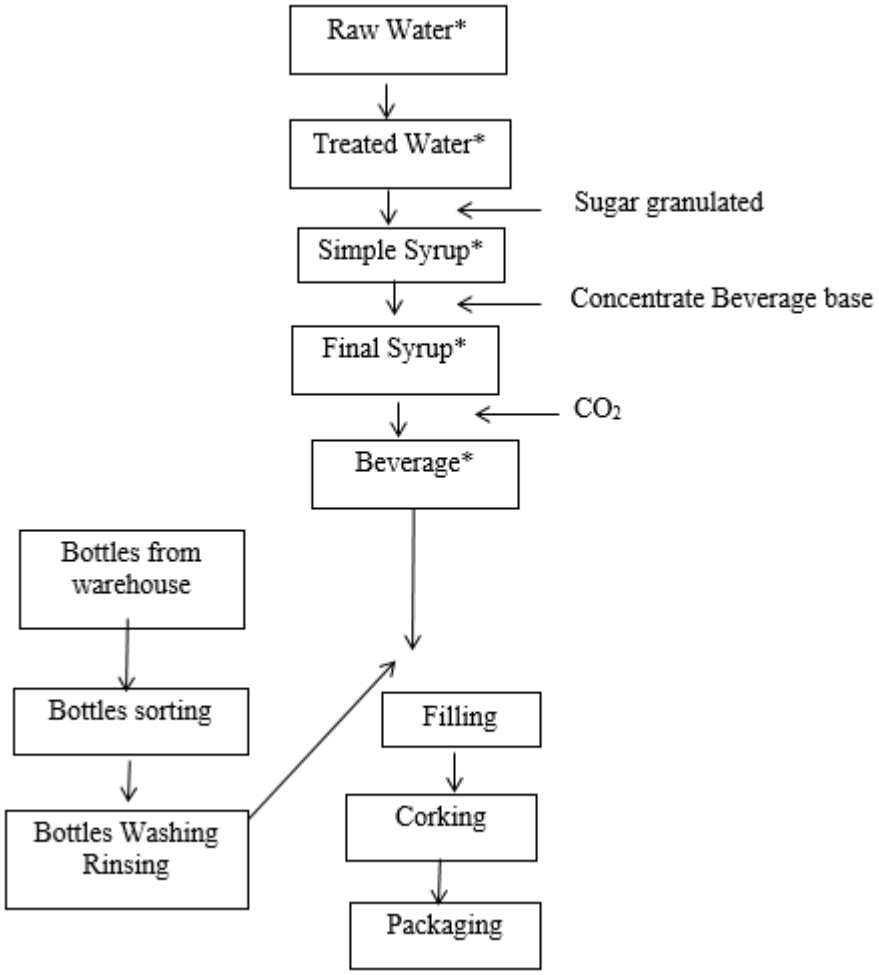


Figure 1: Flow-chart for the production process of Coca-Cola products.

KEY:

- = Sampling Points

Blending (proportioning/ratio): Final syrup: water
Coke - 5.4: 1
Fanta - 4.4: 1
Sprite - 4.4: 1
SBL - 4.0: 1
All others as fanta 4.4: 1 ratio of water to final syrup.

Figure 1 shows the flow-chart for the production process of coca-cola products. The sampling points were the raw water, treated water, simple syrup, final syrup and the beverage (final product) respectively of the production process.

Production Area (Air Sample)

The production environment of Coca-Cola was also sampled to determine the microbial load/population in the production sites. Prepared plates of potato dextrose agar, MacConkey agar and yeast extract agar were allowed to solidify and then exposed t various sites of the production area for about 15 to 20 minutes (Cruickshank, 1975). These sites included; (i) the simple syrup room, (ii) the final syrup room, (iii) the washer II outlet and (iv) filler line II area. After 15 minutes, the plates were covered and incubated at 37°C for 24-48hrs after which the colonies on the plates were counted and reported as Fungi, Coliforms, and bacteria respectively.

Media Preparation

The compositions of the media are shown in the appendix.

Preparation and sterilization of the media were carried out in accordance with the manufacturer's instructions. The prepared media were poured into Petri-dishes for cultivation of the organisms or in Bijou bottles as slants for preservation of isolates. These were allowed to set and solidify before use.

Collection of Samples

Samples were collected aseptically. By sterilizing the mouth of the tap for both sand filtered and carbon filtered samples with 75% ethanol, flamed and allowed to 30 seconds before the water samples were collected (Cowan, 1974). With the aid air r-tight l00ml- Nasco WHIRL-PAK disposable bags.

The granulated sugar samples were collected using pre-sterilized spatula for every sugar sample to be analyzed. The final product (beverage) samples were collected by opening and flaming the mouth of the bottle with the aid of sterile 2m1 pipette.

Isolation of organisms

Ten grams of granulated sugar (10 grams) was dissolved in 90m1 of distilled water. The diluents for this work was 0.1% peptone water, this was prepared by dissolving 0.lg of peptone water in l00mi of distilled water and sterilized by autoclaving at 121°C for 20 minutes (Straka and ltokes, 1976). From this dilution, lml of sugar syrup was added to 9m1 of diluents (0.1% peptone water). lml of this sugar syrup was aseptically collected and a ten fold serial dilution was done using 0.1% peptone water in a set of sterilized test tubes. Using a sterile lml pipette, 0.lml of the dilution were spread into dried Patato dextrose agar, and Yeast extract agar respectively. The plates were labelled according to the dilutions and viable colonies were counted after 24brs incubation at 36°C in a Uniscope Laboratory incubator (Surgifriend Medicals. England). This process applies to all other materials used for this work. This is the standard used for enumeration and is based on the assumption that each viable cell present in the sample will develop into a single colony after incubation of an inoculated medium. The number of colonies is then multiplied by the degree of dilution (dilution factor) to obtain the number of organisms in the original sample.

Bacterial Isolation

Media preparation

Yeast Extract agar was used for the isolation of total viable counts from the samples. Yeast Extracts agar (23g) was dissolved in 1 litre of distilled water shaken to ix and then sterilized by autoclaving for 15 minutes at 121°C (at 15 psi). Appropriate volume was dispensed into sterile Petri dishes and allowed to solidify. Petri dishes were labeled appropriately for easy identification. A ten fold serial dilution was prepared using sterile test tubes which were labeled according to their dilution. 0. lml of each dilution of the sample was aseptically pipetted out and into the Petri dishes in duplicate using the spread plate method. All the were incubated at 35°C in a Memmert incubator (West Germany) for 24hrs.

After which the colonies which developed were counted and then aseptically sub cultured on to fresh nutrient agar medium. From which slants of each of the isolates were made and stored as stock cultures for characterization and identification.

Enumeration of Fungi Preparation of media

Potato Dextrose agar was used for fungal isolation. This was prepared by dissolving 39 grams of potato Dextrose agar in 1 litre of distilled water, swirled to mix and sterilized at 12 1°C for 15 minutes (l5psi). this was dispensed into sterile Petri dishes and allowed to solidify. The Petri dishes were labeled appropriately for easy identification. A ten fold serial dilution of the beverage, final syrup and simple syrup was prepared using sterile test tubes. 0. iml of each dilution of the sample was aseptically pipetted and inoculated into the Petri dishes, this was spread on the solid agar with the aid of a sterile glass rod. All the plates were incubated at 36°C for 24hrs in a UNISCOPE Laboratory incubator. After which colonies were counted and subcultured onto nutrient agar slants for characterization and identification.

Enumeration of Staphylococci Preparation of media

The medium used for isolation of staphylococci was mannitol salt agar. This was prepared by dissolving 111 grams of mannitol salt agar in 1 litre of distilled water, shaken to dissolve completely and autoclaved at 12 1°C for 15 minutes (15 psi). This was dispensed into Petri dishes and allowed to solidify. The Petri dishes were labeled appropriately, for easy identification. A ten fold serial dilution of the water sample and beverage was prepared. 0.1 ml of each dilution of the sample was inoculated into the Petri dishes in duplicate using the spread plate method. All the plates were incubated at 35°C in a Memmert incubator (West Germany) for 24hrs. After which developed colonies were counted and then sub cultured onto fresh nutrient agar medium for identification and characterization.

Enumeration of Coliform Preparation of media

The medium used for coliform isolation was MacConkey agar. This was prepared by dissolving 52 grams of MacConkey agar in 1litre of distilled water, shaken to dissolve completely and then sterilized by autoclaving at 121°C for 15 minutes. This was dispensed into sterilized Petri dishes and allowed to solidify. The Petri dishes were labelled appropriately. A ten fold serial dilution of the water samples was prepared 0.lml of each dilution was inoculated into the Petri dishes in duplicate using the

spread plate method. All the plates were incubated at 35°C in a Memmert incubator (West Germany) for 24hrs after which colonies that developed were counted, and then subcultured onto fresh nutrient agar for identification and characterization.

Characterization and Identification Bacterial Species

Organisms, which grew were subcultured from their respective stock to an appropriate sterile medium and ihUbated at room temperature for 48hrs. Identification and characterization of the isolates were determined after carrying out biochemical and physico-chemical analysis and by comparison with the keys in Bergey's manual of determinative Bacteriology (1994) 9th edition Holt J.G. (ed), Williams and Wilkins Baltimore. Tests carried out included Gram reaction, spore staining, motility test, catalase, methyl red voges proskaeur, indole, oxidase, citrate utilization, urease and sugar (glucose, sucrose, maltose and mannitol) fermentation.

Identification of Fungi Species

Pure cultures of fungi were subcultured onto nutrient agar slant and preserved at 17°C. Macroscopic and microscopic examinations were used for identification. The il isolates were identified using usual observation for colour, staining technique the application of lactophenol cotton blue wet preparation for microscopy. Every fungi has its own spore shape and arrangement on the conidiophores and this was the basis of identification.

Analysis of Physico-chemical Parameters Carbonation

In Carbon dioxide ($C0_2$) production, air and hydrocarbon fuel (oil, kerosene, propane and natural gas) are feed into a burner to produce flue gas consisting of moisture saturated carbon dioxide and nitrogen. The fuel gas is collected in the flue s cooler from where it is led to the $C0_2$ absorber where the carbon dioxide is absorbed in a solution of methanolamine (MEA). The nitrogen gas ($N0_2$) vents to the atmosphere. The saturated MEA carbon dioxide solution is retuned to the stripper where the heat of combustion from the boiler is used to drive off the carbon dioxide gaseous form at low pressure to a specially designed purification train to obtain the highest quality $C0_2$ for the food and beverage industry. It consists of a $C0_2$ wash scrubber and an activated carbon purifier/deionizer. After purification, the carbon dioxide is compressed to 250 lb/sq (17.6kg/cm) with an oil free $C0_2$ compressor and dried in a desiccant type dryer to a dew point of -4F (-40°C) at working pressure. This eliminates freeze-up of the $C0_2$ gas is liquefied at 250lb/sq.cm (17.6kg/sqcm) and I0ºF (-12-2°C) by a self contained packaged refrigeration system. It is then stored in an insulated liquid storage tank and is readily available for its multiple uses.

RESULTS

Physico - Chemical Parameters of Samples

The physic-chemical parameters of samples were analysed, this involves pH, temperature and carbonation. The result is indicated in fig 3.1 and 3.2 below. The microbial loads; total viable counts (TVCs), flingal counts, coliform counts and staphylococci counts of raw water (borehole water) to final product (coke and fanta) were also analysed. Fig 3.3 to 3.7 indicate the various range of microbial loads fhn raw material (raw water and sugar) to final product (coke and fanta).

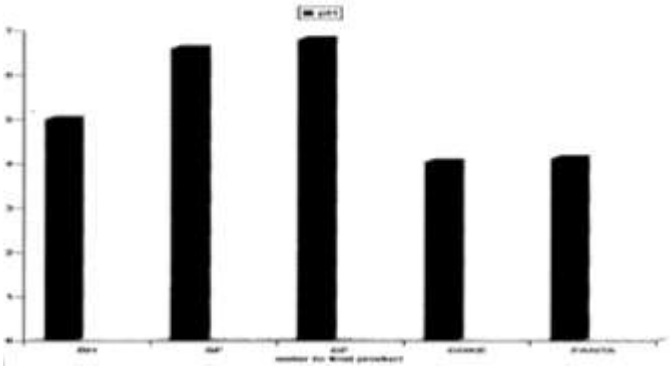
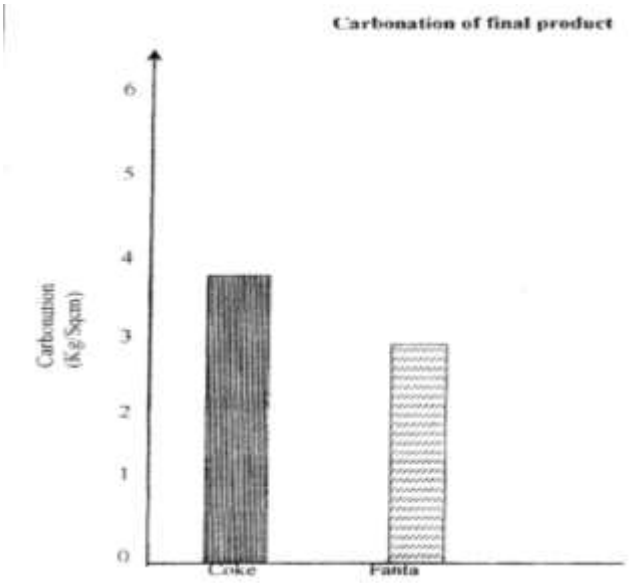


Fig 3.1: Changes in pH values from raw water to final product

Keys

BH = Borehole
SF = Sand filtered water
CF = Carbon filtered water



(Beverage)

Fig. 3.2: Carbondioxide content of final production

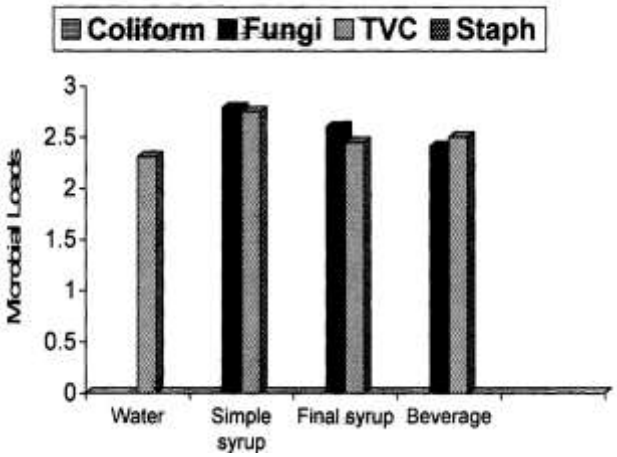
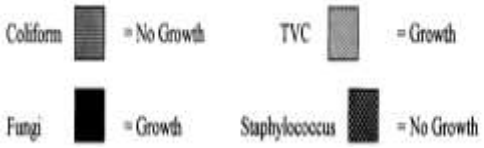


Fig. 3.3: Microbial loads of raw water to final product Coliform

SAMPLES



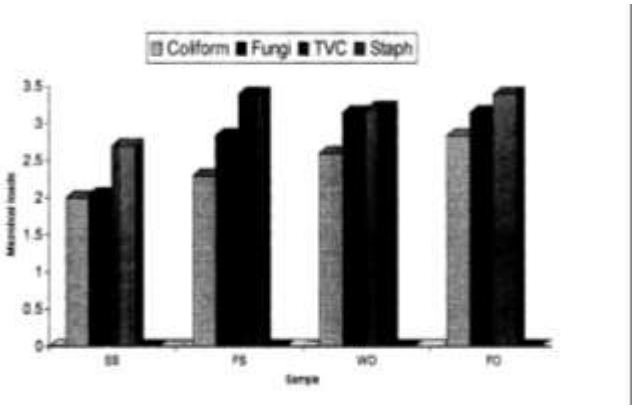


Fig. 3.4: Microbial loads of environmental samples in the production area

Key

SS	=	Simple syrup room
FS	=	Final syrup room
WO	=	Washer II outlet
FO	=	Filler Line II outlet

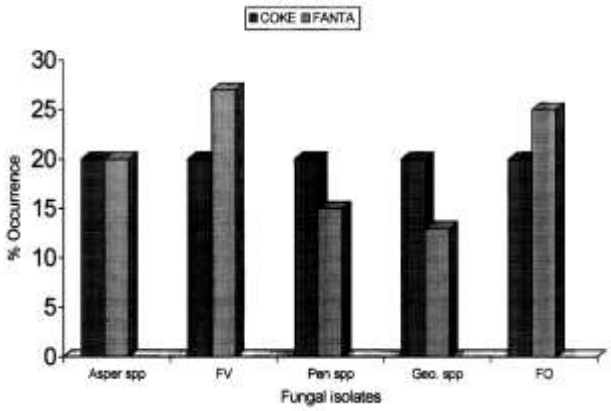
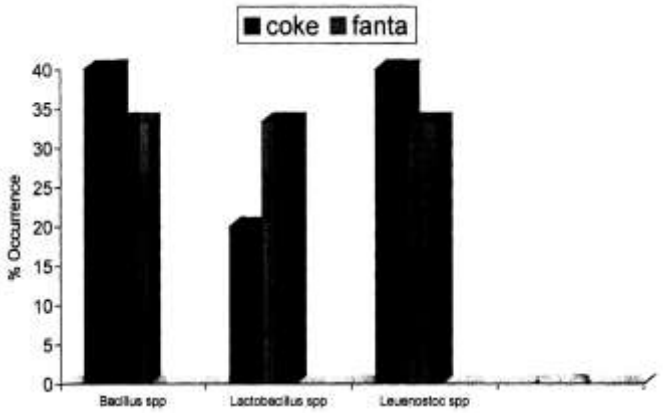


Fig. 3.5: Percentage Occurrence of fungal isolates from coke and fanta sample

Key:

FV	=	*Fusarium verticilloides*
Pen spp	=	*Penicillium spp*
Geo. Spp	=	*Geotrichum spp*
FO	=	*Fusarium oxysporium*



Bacteria

Fig 3.6: Percentage Occurrence of the bacterial genera isolated from coke and fanta

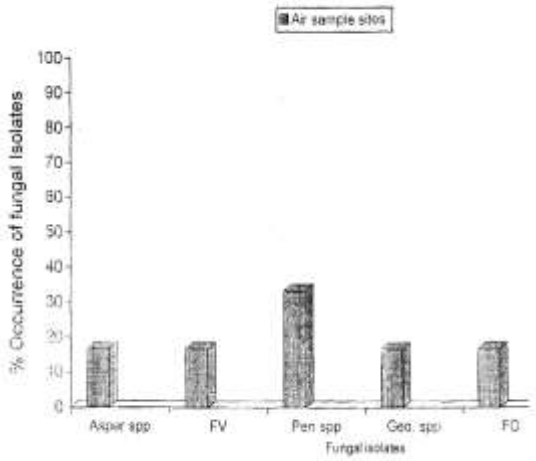


Fig 3.7: Percentage Occurrence of Fungi Isolated environmental Samples

Key:

FV = *Fusarium verticilloides*
Pen spp = *Penicillium spp*
Geo spp = *Geotrichum spp*
FO = *Fusarium oxysporium*

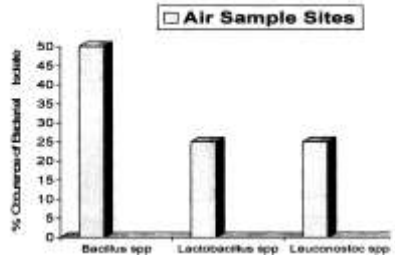


Fig. 3.8: Percentage Occurrence of Bacterial isolates from environmental sample.

Cultural Morphological and Biochemical Characteristics

Tables 10 and 11 show the characteristics of the bacterial isolates from coke and fanta samples which were identified as *Bacillus sp, Lactobacillus sp an. Leuconostoc sp. Bacillus sp* was the most predominant. Table Twelve (12) shows de characteristics of bacterial isolates from Air-Sample sites. These were identified as *Bacillus sp, lactobacillus sp* and *leuconostoc sp,* respectively. Tables 13 and 14 shows the characteristics of fungal isolates from coke and fanta samples; these were identified as *Aspergillus sp, Penicillium sp, Fusarium sp,* and *Geotriclum sp* with *Fusarium sp,* more predominant. Table 15 shows the characteristics of fungal isolates from air-sample sites amongst which were *Geotriclum sp, Penicillium sp, Asperigilus sp* and *Fusarium sp,* with *Fusarium sp* and *Penicillium sp* more predominant.

Table 10: Cultural Morphology and biochemical characteristics of bacterial Isolates from coke samples

Isolate cods number	Cultural characteristics	spores	Catalase	Citrate	Starch	Motility	Indole	oxidase	MR	VP	Coagulase	Grsams reaction	H_2S Prod.	Nitrate oxidastion	**Sugar fermentation**				Urease		Ga1s product	Nitrate reactio	Identified genera
															Glusoe	Maltose	Mannitol	Sucrose	Lactose				
A1	Irregular undulate, Grenated cream and opaque	Rods	+	+	+	+	+	+	+	+	+	+	-	-	A	A	A	A	-	-	+	+	*Baccillus spp*
A2	Circular raised, entire cream, milky rough and translucent	Rods	-	-	-	-	-	-	+	-	-	+	-	-	-	-	A			-			*Lactobailliu s sp*
A3	Yellow colonies	Shoft rodish chain	-	+		-	-	-				+	-	-	A				-		-		*Leuconost oe spp*
A4	Milky white colonies	Cocci	-	-			-	-				A		A		-			-			+	*Leuc+onos toe spp*
A5	Mucored colonies	Strait rods in pairs	+	+	+	+	-	-	-	-	-		+	-	A	-	A	-	+		-	+	*Bacillus spp*

KEY: **+** Positive reaction A = Acid Production
- Negative reaction **+** - = Weak reaction

Table 11: Cultural Morphology and biochemical characteristics of bacterial Isolates from Fanta samples

Isolate cods number	Cultural characteristics	Cell Morphology	Spores	Catalase	Starch	Motility	Indole	Oxidase	MR	VP	Coagulase	Grsams	H2S Prod.	Nitrate oxidation	Glucose	Maltose	Mannitol	Sucrose	Lactose	Urease	Gas Production	Identified genera
B1	Milky colonies	Cocci	+	-	+	-	-	-	-	+					A	A	-	-	-	-	+	*Lactobacilliu s spp*
B2	Milky smooth colonies	Strait colonies	+			+	+	-	-	-	-	-	-	-	A	-	A	A	-	-	+	*Bacillius spp*
B3	Circular, raised entire, cream milky rough translucent	Rods	+	-	-	-	-	-	-	-	+	-	-	-			A	-	A	-		*Lactobacilliu s spp*

KEY: + = Positive reaction; - = Negative reaction; A = Acid Production; + - = Weak reaction

Table 12: Cultural Morphological and biochemical characteristics of bacterial Isolates from air Sample sites

Isolate cods number	Cultural characteristics	Grain reaction	Spores	Catalase	Citrate	Starch	Motility	Indole	Oxidase	MR	VP	Coagulase	Grams reaction	H_2S production	Nitrate	Glucose	Maltose	Mannitol	Sucrose	Lactose	Urease	Gas production	Identified genera
C1	Orange smooth colonies	Strait rods in pairs	+			+				-	-		-	-	-	A	-	A	-	-	-	+	*Bacillius spp*
C2	Circular, raised cream milky rough translucent	Rods	+	-	-	-	-	-	-	+	-	-	-	-	-	A	-	A	-	-			*Lactobacill ius spp*
C3	Milky colonies	Cocci	+	-	+	-		-	-	-	-	+			-	A						+	*Leuvonost oc spp*
C4	Mycoid colonies	Rods in pairs	+	+	+		+	-	-	-	-	-		-	-	-	A	-	A		-	+	*Bacillius spp*

KEY: + = Positive reaction; - = Negative reaction; A = Acid Production; + - = Weak reaction

Table 13: The Colonial morphology and microscopic features of fungi isolated from coke sample

Isolate code	Reverse/Back	Front	Microscopic Description	Identified fungi
A1	Black	Dense, almost mucoid, with small thread like mycelia	Black spores conidiophores upright, bearing philalides at the apex, globuse coniclia	*Aspergillus spp*
A2	Yellow with lobates edge	Green, yellow edge with yellow droppings by the side	Ovoid hyaline coloured condidia, conidiophores arising from a singly branched mycelium near the apex	*Penicillium spp.*
A3	Pink	Fluffy	Branched conidiophores, some conidia were in chains	*Fusarium verficilloides*
A4	Whitish black collony	Concedric smooth and feathery	Advancing mycelial growth	*Geotrichum spp*
A5	Whitish ,	Whitish fetfy collony	Branched conidiophore conidia smooth or rough walled in chains or pairs	*Fusarium oxysporium*

Table 14: The Colonial morphology and microscopic features of fungal isolates from Fanta sample

Isolate code	Reverse/Back	Front	Microscopic Description	Identified fungal genera
B1	Pink	Loose fluffy pink mycelia	Curved multiseptate macro conidlia	*Fusarium verficilluids*
B2	Dark	Sandy brown dusty spreading	Conidiophores upright simple terminating in a globules swelling bearing philalides at the apex	*Aspergillus spp*
B3	Yellow with white edge	Green, yellow with yellow droppings by the side	Ovoid hydaline coloured conidia and conidiophores branched near the apex	*Penicillium spp*
B4	Whitish black colony	White concentric feathery	Advancing mycelial growth, hyphae Septate, dichotomously branched (forked) conidia cylindrical in chains and erect	*Geotri chum candidum*
B5	Whitish	Whitish fatty colony	Branched conidiophores conidia smooth in pairs or chains	*Fusarium oxysporium*

Table 15: The Colonial morphology and microscopic features of fungal isolates from environmental sample sites

Isolate code	Reverse/Back	Front	Microscopic Description	Identified fung genera
C1	Whitish	Whitish baick coffony concentric smooth and feathery	Hyphae Septate dichotomously branched (forked) conidia cylindrical chains and erect	*Geotrichum spp*
C2	Yellow with white edge	Green, yellow with yellow dropping by the side	Ovoid hyaline coloured comdia and conidiophores branched near the apex	*Penicillium spj*
C3	Whitish	Whitjsh coffony whitish raised characteristically restricted	Brush like appearance, Septate hyphae with branched conidiophores having metulae	*Penicilliurn spp*
C4	Blackish	Blackish grey colonies blackish coffoney (threadlike)	Sepetate hyphae with branched conidiophores bearing resides that produce comdia	*Aspergillus spp*
C5	Pink	Pink colony fluffy velvety appearance	Branched conidiophore, comdia smooth or rough walled in chains or pairs	*Fusarium verficilloides*
C6	Whitish	Fetfy coffony	Branched conidiophore, comdia smooth or rough walled in chains or pairs	*Fusarium oxysporium*

The fungi were identified as *Fusarium sp, Penicillium sp, Aspergillus sp* and *Geotrchurn spp,* with *Fusariurn spp* more predominant.

DISCUSSION

The intrinsic characteristic and the processing parameters as well as the distribution practices of a particular food products have considerable impact on the microbial flora and its shelf stability. The heterogeneity of the soft drink (coke and fanta) samples is probably related to poor quality of raw materials, inadequate manufacturing practices as well as compromised quality control measures. For instance, sub-standard raw materials including water were reported to be responsible for quality deterioration of most soft drinks in Nigeria (SHdhar *et al*., 1986). It is also striking that most of the raw materials used are imported and are often subjected to abusive handling and storage practices which adversely affect the microbial quality of soft drinks (Effiuvwevwere and Chinyere, 2001).

CONCLUSION

The different microbial distribution in the two brands and the air -sample sites may in part be attributed to difference in the chemical composition of the products. It is known that coke brand contains caffeic acid, a plant phenolic which has antimicrobial property while fanta brand does not (as indicated on the crown). However, dominance of Bacillus spp (50% and more) in the two brands and air sample sites could be associated with their prevalence in soft drink factories (Panezai, 1978). Additionally, as spore formers, their heat resistant properties and adaptive responses to stress conditions may have influenced their survival, recovery and dominance (Setlow, 1994).

The lactobacillus species which accounted for 20% and more of the total viable count were the next major group of isolates. As common contaminants, they are very acidic and carbon dioxide tolerant and can grow well in the presence of high sugar content (Effiuvwevwere and Oye lade, 1991). Their occurrence confirms their association with such acidic environment and foods. The occurrence of Leuconostoc species (20% and more) indicates their presence in the inadequately cleaned and sanitized food utensils and equipments used during processing of these drinks (Frazier and Westhoff, 1978).

The greater diversity of the fungal population isolated from fanta samples suggest the impact. of the differential intrinsic properties (formulations) of each product for example, ascorbic acid used as preservation for soft drink is known to inhibit yeasts and moulds but are less effective at p11 4.0-6.5 (Frazier and Westhoff, 1978). However, the pH values obtained in the present work were in the range of 4.04-4.11.

The incidence of *Asprgillus species* occurring at the same percentage as *Fusarium spp* (16.7% - 20.0 %) may be due to their prevalence in soft drink environment (Odunfa, 1987). Moulds can grow over a wide range of pH values (2 to 8.5). Mold spores are small in size resistant to drying and large numbers per plant are usually present in air and this may have led to a high occurrence in the fanta and coke samples than the

yeasts. In addition, moulds in general utilize many kings of foods ranging from simple to complex (Effiuvwevwere and Chinyere, 2001).

REFERENCES

Bergey's Manual of Determinative Bacteriology, (1994) 9th ed. bit J. G. (ed). Williams and Wilkins Baltimore Maryland. 30, 1-350.

Bissessur, J.K, Permaul, and B. Odhar (2001), Reduction of patulin during apple juice clarification, *Journal of Food Protection,* 64 (8) 337-339.

Blackburn, dive de W. and Peter J. M. (2002) Food borne pathogens; Hazards, Risk Analysis, and Control. CRC press, Washington DC.

Codex Alimentarius Commission (1997). Hazard Analysis and critical control point (JIACCP) system and Guidelines for its application Annex to CAC/RCP, 1969, Rev. 3, FAO, Rome.

Cruickshank, R, J.P. Duguid, B. P. Marion and R. H.A Swain, (1982). Medical microbiology, 12th ed. Vol 1.1: The practice of medical microbiology. Longman group, London, pp 323-40 1.

Cruickshank, V., Pill, S., Williams, J.R., Mambridge, C.P., MacDonald, A and Elimer, S. (2022). Exploring the everyday Philosophies of general test Primary School teacher delivery of health literacy education. Curriculum studies in Health and Physical education. https://doi.org/10.1080/25742981,2022.2059384.

Donald, W Anderson, M Mary, K. and Shawn A. K (2001) Analysis of Hazard Analysis Critical Control Point (HACCP), RT1 44.

Efiuvwevwere B. J. O. and Oyelade J. A (1991). Biodeteriorative and physic-chemical changes in modified Atmosphere Packed oranges and the microbial quality of preserved juice — Tropical Science. 13:325-333

Efiuvwevwere B. J. 0. (2000). Microbial spoilage agents of tropical and assorted fruits and vegetables (Para graphics publishers) PP. 11 -38.

Efiuvwevwere, B. J. 0 and Chinyere M. (2001). The microbiology and Deterioration of soft Drinks subjected to two different marketing conditions, Global Journal of Pure and Applied Sciences 7, (No. 1): 43-47.

FAO (2002). Guidelines for small scale fruit and vegetable service processors. Food and Agric Bull, 12, 7-10.

ILS, Europe (2004), Food safety objectives: Role in microbiological food safety management. Mike stringer (Author). ILSI Europe Report series. International life sciences institute. Brussels. ISBN I-57881-175- 9.

ILSA Europe (2001), principles of risk assessment of food and drinking water related to human health. Diane Ben ford (Author). ILSI Europe conase monograph series. International life sciences institute Brussels. 1- 57881-124-4.

International Commission on Microbiological specifications for foods (2002). Microorganisms in foods, Book 7, microbiological testing in food safety management Kiuwer Academic! Plenum, NY,

International Commission on Microbiological specifications for foods (2002). Microorganisms in foods, Book 7, microbiological testing in food safety management Kiuwer Academic! Plenum, NY,

Jackson, L.S. Tina, B. B., Susanne E. K., Kirk T. T. (2003) Applied Quality storage and Washington Treatments Journal of food protection. 66, (4), 1154-1157.

Notermans, S and Heuvelman, C.J. (1983). Combined Effect of Water Activity, pH and Sub-Optimal Temperature on Growth and Enterotoxin production of staphylococcus aureus. *J Food sci*., 48:1832-1 835.

Panezai, A.K. (1978). Microbiology. In: Development in soft drinks Technology. 1. Green, L. F (ed). 209 — 227, applied science Publishers ltd. London.

Setlow, P. (1994). Mechanisms which contribute to the long term survival of spores of Bacillus Species Journal of Applied Bacteriology symposium supplement. 76: 495-605.

Sridhar, M.K.C, Omishakin, M.A., and l3amiiieke, A. 0. (1986). Shelf life of foods and Beverages and the Quality Control Practices in Nigeria. Proc. 4th mt. flavor conf. Rhodes, Greece, 23-26. Charalambous, (Ed) Elsavier Science Publishers, Amsterdam.

Suttajit, M. (2002) Prevention and control of mycotoxin in food Grains American journal of public Health. 85 (8), 1142-1145

Tompkin, R. B. (2002). Control of pathogenic organisms in the food processing environment. Journal of Food Protection. 65. 4: 709-725.

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