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Microbiological and Physico-chemical Evaluation of Some Non-alcoholic Beverages

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Abstract: The microbial and physico-chemical quality of our commercially produced non-alcoholic beverages-Ginger beer, Soya milk, Soborodo drink and kunun-zaki-were investigated. The organisms isolated included lactic acid bacteria like Lactobacillus, *Streptococcus* and *Leuconostoc.* Other bacterial isolates were *Staphylococcus, Bacillus* and *Pseudomonas* while the fungi were *Penicillium, Aspergillus, Trichoderma, Candida* and *Saccharomyces.* Studies were also carried out on the sensory evaluation and shelf-stability during storage. A combination of pasteurization and refrigeration was found most suitable for prolonged shelf life and consumer acceptability. Chemical analysis showed that the major food components were retained, though some toxic elements were detected like Cadmium and-Chromium even above the permissible levels.

Key words: Non alcoholic beverages, bacteria, pasteurization, refrigeration, toxic elements

Introduction

Non-alcoholic beverages play a very important role in the dietary pattern of people in developing countries like Nigeria. They are regarded as after meal drinks or refreshing drinks during the dry season in rural and urban centres. Most of these beverages are made up of about 90% water, sugar, flavouring agents and sometimes preservatives.

Plants parts have constituted the main raw materials in these beverages. Soybeans are used for the production of soyamilk, rhizome of ginger plants for ginger beer, sorghum grains for the beverage 'kunu-zaki' and the Calyces of the Roselle plant (*H. sabdariffa* var *sabdariffa*) for the extract called the 'soborodo' drink. All these plants are commonly grown in Nigeria especially in the northern part of the country. The non-alcoholic nature of these beverages makes them to be readily consumed by Christians and Muslims alike as a substitute for alcoholic ones (Onuorah *et al*., 1987).

They are also very nutritious and of medicinal value. Soya milk contains 26.25% protein, 22.24% oil, 21.55% total solids, 4.55% Ash, 24.4% carbohydrate (Aderiye, 1990). Ginger powder has 5.21% ether extract, 61.21% carbohydrate, 8.89% protein. 8.47% ash and 1.70% crude fibre (Meadows, 1988). The aroma constituent is contained in the essential oil while the pungent nature is found in the Oleoresin. The sorghum gruel Kunu-zaki has about 76.3% starch, 11.6% proteins, 3.3% fat 1.9% fibre and 1.3% ash along with a wide array of amino acids (Lichtenwalner *et al*., 1979). The additive that is used is sweet potatoes, it contains essential amino acids and is a rich source of vitamins.

The Roselle plant is said to be antiseptic, aphrodisiac, astringent, diuretic, and purgative among many uses. The drink made by placing the calyx in water is said to be folk remedy for cancer. This red beverage is also used in jams, tea, ice cream, pies, deserts, jellies, sauces and wines in some part of Europe and the West Indies The preparation of these beverages has become a common technology in many homes in the rural communities and more recently in the urban areas. Many women have developed the skill and commercial production has helped to alleviate poverty among the people. In Nigeria, many women have been able to set up small-scale commercial production of these beverages due to support from the government through the poverty alleviation scheme.

These beverages are however highly prone to microbial deterioration if not adequately stored. A large number of lactic acid bacteria, coliforms, molds and yeasts cause spoilage as they can use the carbohydrate content for fermentation processes producing undesirable changes in them. The products of fermentation like acids, alcohols, diacetyls affect the organoleptic properties of foods. The sugar used as sweetening agent also contributes to these changes (Inajor, 1995).

This study was undertaken to estimate the physio-chemical and microbiological quality of these beverages so as to determine the most effective mode of storage and consumer acceptability.

Materials and Methods

Collection of sample: The plant materials, soybeans, sorghum grains, ginger rhizome and calyx of *Hibiscus sabdariffa* L. were purchased from markets (Sabo, Mile 12, Tejuoso, Sangrause and Iddo) in Lagos metropolis.

Preparation of samples

Soya milk: The beans were sorted out, rinsed in tap water and 100g of it was placed in 500mls of boiling

water containing a few drops of 0.5% $NaHCO_3$ which aids softening. Boiling was done for 15 mins. The blanched beans were manually dehulled and milled in a Stephan milling machine (Gallenkamp model) before being homogenized using a Moulinex electric blender containing 500mls of water. The slurry was filtered with a six fold finely woven cloth to obtain the clear filtrate which is the milk product.

Sorghum based beverage (kunu-zaki): The sorghum grains were dehulled, sorted out and rinsed in tap water. About 700g were steeped in tap water for 12 hrs. The grains were drained and malted (i.e covered and allowed to germinate) for 24 hrs. Along side, 300g of grains were steeped in water. The soaked and malted grains were mixed and thoroughly washed. To this was added 30g peeled ginger and 300g peeled sweet potatoes. Pepper and cloves may be added. This was wet milled and made into a paste. Five litres of clean tap water was allowed to boil and poured into the paste and mixed. The gruel was allowed to cool for about 8 hours (usually overnight). The resulting mixture was wet sieved using a wire mesh sieve or a clean muslin cloth. The filtrate is the kunun. Sugar was added as a sweetener according to preference (Adeyemi and Umar, 1994).

Ginger beer: The ginger rhizomes were placed in a clean bowl, washed to remove adhering soil debris. The roots were peeled manually, washed and 1kg portion was diced into cubes. The cubes were placed in a blender cup containing 500mls of water and homogenized. The resulting pulp was added to 3,000mls (3litres) of boiling water and allowed to boil for three minutes. It was then left to stand for three hours and 100g of fresh limejuice and cloves were added as flavouring agents. The juice was filtered through a muslin cloth. The filtrate was allowed to stand for one hr and the clear liquid was decanted. Nine litres of water containing 500g of sugar was added as a sweetener.

Roselle drink 'soborodo: One hundred grams of dried calyx were sorted out and rinsed in tap water. It was added to 1,500mls of water and boiled for 3-5 minutes. It was left to stand for about 30 minutes. It was sweetened with sugar and can be served chilled or hot as tea Flavouring agents like fresh pineapple juice, lime juice or artificial flavouring like strawberry or vanilla could be added depending on individual taste.

Microbiological quality of sample: An aliquot of 1ml of the freshly prepared samples or from the serially diluted samples was placed on Nutrient Agar, MRS Agar and Potato Dextrose Agar using the spread plate method for bacterial and fungal isolation respectively. The nutrient agar plates were incubated at 37°C for 24 hrs for bacterial isolation. MRS agar plates were incubated anaerobically for *Lactobacillus*. Fungi were isolated at 28-30°C for 3-5 days on PDA. Pure cultures of each of the isolates were prepared and identified using morphological and biochemical characteristics.

Storage studies: The different samples were stored by dispensing 20ml portions into clean sterile plastic bottles of 25ml capacity. They were divided into three groups, some were kept in the refrigerator (4°C), some were pasteurized at 72°C for 15 minutes and kept at 4°C while the other were pasteurized in a similar manner and left at ambient temperature (28 ± 2°C). The fresh samples kept at ambient temperature (unpreserved) served as controls.
All the samples were monitored for microbial quality, p^H changes and sensory evaluation was carried out every 48hrs for a period of thirty days.

Sensory evaluation: A fifteen-man panel was set up to evaluate the organoleptic qualities (i.e. smell, taste and appearance) of the stored samples. Questionnaires were distributed to each of the panelists. The paired comparison test was used to analyze the responses of the panelists with regard to their preference for the samples (Esan and Okafor, 1995).

Physio-chemical analysis: The following physio-chemical parameters were determined using standard analytical methods, pH, titratable acidity, total solid, specific gravity, and viscosity. The presence of toxic metals was investigated using atomic absorption spectrophotometer, for lead, cadmium and chromium.

Phytochemical screening: Soborodo 'drink, an extract from calyx of the Rosdle plant 'was qualitatively tested for anthroquinones, free and combined glycosides, tannins, saponins. Odebiyi and Sofowora (1978) Tannins and total polyphenols were quantitatively estimated by modified vanillin-hydrochloric acid method of Price and Butler, 1977 and by the method of McGrath *et al.,* 1982 respectively.

Results

Microbiological quality: The sorghum gruel (kunu-zaki) was characterized by the presence of *lactic* acid bacteria, that is *Lactobacillus* and *Streptococcus* while *Penicillium* and *Aspergillus* were the fungal isolates. Ginger beer had some lactic bacteria i.e *Lactobacillus* and *Leuconostoc* along with *Bacillus, Staphylococcus, Candida and Saccharomyces*. The soyamilk had only *Pseudomonas* species while the Roselle drink (Soborodo) had no bacterial isolate but contained *Aspergillus* species and *Trichoderma* species. Refer to Table 1.

Table 1: Isolated microorganisms in different beverages

Beverage	Isolated microorganisms
Kunu-Zaki	*Lactobacillus*, *Streptococcus* *Aspergillus Penicillium*
Ginger beer	*Lactobacillius, Leuconostoc, Bacillus, Staphylococcus, Candida* and *Saccharomyces*
Soyamilk	*Pseudomonas*
Soborodo	*Aspergillus, Trichoderma*

Storage studies: Microbial population decreased considerably when the samples were pasteurized and refrigerated (Fig. 1) and shelf life ranged from 6 days for Soya milk, 14 days for Ginger beer to 24 days with 'kunu-zaki'. Pasteurization was more effective than refrigeration and spoilage was very pronounced at ambient temperatures. Shelf life at ambient temperatures for all the beverages was between 1 to 2 days.

P^H Changes: All samples had pH of about 6.2 immediately after preparation. The samples stored at ambient temperature became increasingly acidic during storage. This trend was not so pronounced in the pasteurized and refrigerated samples. Refer to Fig. 2.

Sensory evaluation: There was a high percentage for the pasteurized and refrigerated samples when compared with pasteurized or refrigerated samples. The lowest percentage recorded was for the unpreserved samples. Refer to Fig. 3.

Physico-Chemical Analysis: The physico-chemical parameters of Kunun-saki and Soyamilk showed that the following parameters titratable acid, total solids, Viscosity and specific gravity are higher in kunu-zaki than in Soya milk. Lead was not detected in both drinks but the concentrations, of Chromium and Cadmium were slightly higher in Soya milk than in Kunu-zaki (Table 2).

Table 2: Physico-Chemical Analyses

Parameters	Kunu-Zaki	Soyamilk
P^H	5.79	6.90
Titratable acid	2.30%	1.45%
Total solids	131.33g/l	68.54g/l
Specific gravity	1.0426	1.0249
Viscosity	7,247cts	2.093 cts.
Metals		
Lead	Not detected	Not detected
Cadmium	0.015mg/l	0.028mg/l
Chromium	0.074mg/l	0.075 mg/l

Photochemical screening: The deep colouration of Soborodo drink is due to the presence of anthraquinone and polyphenols (Table 3a and 3b).

Phytochemical screening of soborodo drink

Table 3a: Qualitative Screening

Test	
Anthraquinone	
Free anthraquinone	+ ve
Combined anthraquinone	+ ve
Glycodises	+ ve
Alkaloids	+ ve
Tannins	+ ve
Saponins	- ve

Table 3b: Quantitative Screening

Tannins	
Catechin equivalent	17.5mg/g
Polyphenols	
Tannic acid equivalent	35 mg/g

Discussion

Food remains an important requirement for sustenance of life either for plants or animals. The demand therefore, cannot be overemphasized. In Nigeria, a wide variety of foods are prepared through indigenous technology from many plant products. They include non-alcoholic beverages like those ones used in this study. These beverages are prepared in the homes usually under unhygienic conditions and thus are prone to contamination by the micro flora of the raw materials and of the utensils. The micro flora of the finished product however depends on the processing and storage conditions. Lactic acid bacteria such as *Lactobacillus*, *Leuconostoc* and *Streptococcus* species were isolated especially in the cereal gruel. This is not surprising as these organisms thrive in medium rich in fermentable substrates such as sugars which can be degraded with production of acids. This is responsible for the drop in P^H. Their thermoduric nature ensures survival at pasteurization temperatures and hence their presence in the treated samples.

Bacillus species are spore formers whose spores could survive high temperatures of processing. Rhodes and Fletcher (1966), found that *Bacillus* and *Lactobacillus* species were readily found in foods of low acid content like juices and beverages where they produce organic acids.

Staphylococcus and *Psuedomonas* species were possible contaminants from handlers and utensils used especially after the processing, as they are mesophiles though some *Pseudomonads* are spoilage organisms at refrigerated temperatures.

The presence of *Aspergillus* and *Penicillium* was not surprising as they are common spoilage organisms of carbohydrate foods and storage micro flora of many cereals (Rhodes and Fletcher, 1966). The high survival rate of their spores could also explain their role. The yeasts, *Candida* and *Saccharomyces* probably played a

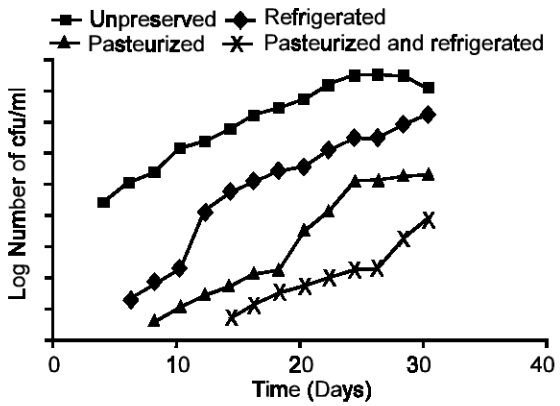

Fig. 1: Mean changes in the microbial populations isolated from samples during preservation under different physical condition

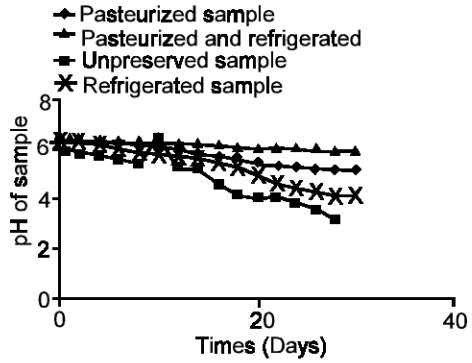

Fig. 2: Mean changes in the pH of samples during preservation under different physical conditions

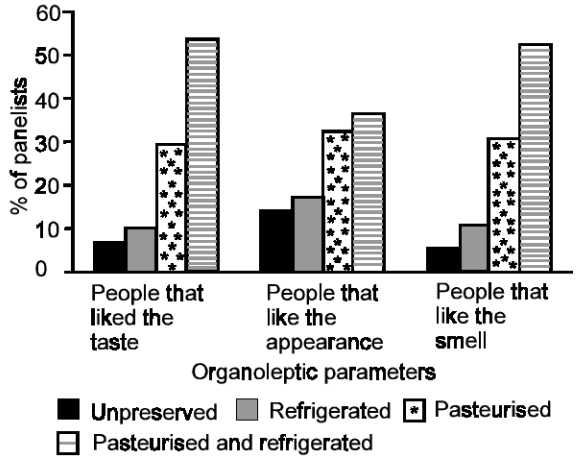

Fig. 3: % of panelists for various samples

secondary role. *Saccharomyces* species convert starch and dextrins to glucose that is fermented to ethanol and CO_2 while *Candida* species produce esters and phenolic off flavours when present in food (Uraih, 1997).

The processing and storage condition influence the presence of pathogenic and spoilage organisms in food. The use of high temperatures like pasteurization destroyed most mesophilic organisms while low temperature storage controls the proliferation of those present. This must be responsible for the low microbial counts in the pasteurization and refrigerated samples. The organoleptic qualities, taste, smell and physical appearance were not altered by pasteurization. The storage at refrigerated temperature further enhanced consumer acceptability from the sensory evaluation studies. This agrees with the report of Beaton and Henry, 1964 that temperature is connected with the sensation of heat and cold, this affects individual preference for the temperature at which such foods were served. It is generally believed that chilled drinks like wine taste better than non-chilled ones. The most effective mode of preservation is combined pasteurization are refrigeration as shelf life was prolonged as the samples were most favoured by panelist.

The physico-chemical analysis of kunun-zaki and soya milk showed that the levels of the toxic metals cadmium to be 0.015 and 0.028 mg/l which is higher than WHO permissible levels of 0.01 mg/l. Chromium was 0.074 and 0.075 mg/l (WHO permissible level 0.05 mg/l). Their toxic metals are carcinogenic affecting the kidney and liver. The presence of these metals could be accounted for either from the water or from the soil used in planting the grains especially if phosphate fertilizer is used. A similar result was obtained for kunun baule in Bauchi Northern Nigeria (Wufem *et al.*, 2000).

Phytochemical screening of the Soborodo drink showed the presence of anthraquinone, glycosides, tannins and polyphenols. These compounds are known to complex with protein in the body (Osuntogun, 1984; Afolabi *et al.*, 1988).

Thus this Soborodo drink should be taken with caution because of the side effects of polyphenols. Also Kunun saki, Soya milk and ginger drinks should be prepared under hygienic conditions to prevent the toxic metals from accumulating in the body.

Industrial production should be highly recommended as pasteurized, bottled and chilled drinks could not only alleviate the longing for fluid intake in warm tropical climate but would also provide a cheaper and more nutritive drinks than the sugar laden fizzy drinks in the market. Weisberg (1976) stressed that food products should be developed to improve the nutrition in the developing world and they must be relatively cheap and nutritionally standardized.

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