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THE MICROBIOLOGY AND DETERIORATION OF SOFT DRINKS SUBJECTED TO TWO DIFFERENT MARKETING CONDITIONS

B. J. O. EFIUVWEVWERE and M. CHINYERE

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ABSTRACT

The microbial quality and successional changes in two brands (A and B) of soft drinks held under two marketing conditions (ambient *ca* 28° C and open-air *ca* 34°C) were investigated using spread-plate technique. Heterogeneous flora was isolated from the two brands but varied with brand. *Pediococcus* spp. occurred in brand A but not in brand B. Much higher incidence (30%) of *Staphylococcus* spp. was observed in brand B as compared with 10% in brand A. *Bacillus* spp. were the most predominant bacterial group found in both brands while *Aspergillus* spp. and *Cladosporium* spp. dominated the two brands. Incidence of *Bacillus* spp. in samples held in open-air was remarkably increased by approximately 103% in brand A but tended to remain unchanged in brand B. Exposure of samples to the two conditions resulted in about 2-fold increase in *Lactobacillus* spp. in brand B on day 14 and decreased thereafter but decrease occurred in brand A after initial increase. Initial increase in *Staphylococcus* spp. occurred in both brands but became non-detectable in samples held under ambient condition as opposed to low incidences in samples subjected to open-air. Dramatic changes also occurred in the mycoflora; *Arthrinium* spp. were not isolated until day 14 and higher incidences occurred in samples held in open-air. *Saccharomyces* spp. were undetected in the samples until day 21. Approximately 13- and 9-fold increases in total aerobic bacterial counts were observed in brands A and B samples held in at 34° C respectively. Higher fungal populations occurred in both brands subjected to open-air with brand B samples showing the higher population. The diversity of the microbial flora and the phenomenal changes in incidences of *Bacillus* spp. coupled with prevalence of *Aspergillus* spp. in the open-air -held samples could pose health risks. Adoption of adequate quality control measures and distribution practices would likely reduce the microbial diversity and deterioration (characterised by sedimentation) due probably to *Bacillus* spp. and *Saccharomyces* spp. Consumption of these products is not advised after 14 days of exposure to the marketing conditions studied in this work.

KEYWORDS: Soft drinks, microbial composition, deterioration, marketing conditions.

INTRODUCTION

Soft drinks (non-alcoholic carbonated beverages) are popular worldwide (Hollingsworth 1997). In Nigeria, they are produced and consumed throughout the country and the soft drink industry is probably the most viable among the food industries. Several brands of these products including Coke, Fanta (orange), Pepsi, Sprite, Seven-up are produced on a large scale by different bottling companies.

The raw materials and composition of soft-drinks vary. However, sugar (sucrose) is the approved sweetening agent in Nigeria. The composition of sugar-sweetened non-alcoholic carbonated beverages in Nigeria is as follows: water (90%), sugar (9-14%), carbon dioxide 3.5-4.0 gas volumes and other minor but important ingredients such as citric acid, phosphoric acid, sodium benzoate result in a final pH of 2-5 (Ibiyemi et al. 1987).

Various studies on the microbial contamination of soft drinks have been reported (Sridhar et al. 1986; Odunfa 1987; Vanderzant and Splittstoesser 1992). Recently, Mossel et al. (1995) emphasized that the distribution practices of foods and beverages are as important variables as the production processes; hence they constitute part of the hazard analysis critical control points concept. Similarly, the need for storage tests (or ' shelf-life studies') to provide information on expected microbial changes following distribution until consumption has been highlighted (Notermans and Mead 1996). However, literature is deficient on information concerning the effect of distribution practices and marketing conditions on microbial quality of soft drinks. Several complaints of spoilage and questionable safety of soft drinks are frequent in Nigeria and they are of public concern (Sridhar et al. 1986). Therefore, the present study was carried out to investigate the microbiological composition and the evolution of the microbial flora of two most popular brands of soft drinks in Nigeria (and in many other countries) subjected to tropical distribution and marketing conditions. This would provide information on the initial microbial composition and the succession of the flora as well as the deterioration and safety potentials.

B. J. O. EFIUVWEVWERE, Department of Microbiology, Food & Industrial Division, University of Port Harcourt, Port Harcourt, Nigeria
M. CHINYERE, Department of Microbiology, Food & Industrial Division, University of Port Harcourt, Port Harcourt, Nigeria

Table I. Evolution and incidence (%) of different microbial flora of two brands of soft drinks subjected to two different marketing conditions

	Holding time (days) and marketing conditions															
	Ambient condition (*ca* 28°C)								Open-air (*ca* 34°C)							
	0		7		14		21		0		7		14		21	
Brands	Brands		Brands		Brands		Brands		Brands		Brands		Brands		Brands	
Bacterial flora	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Bacillus spp.	40	50	50	38	62.5	45.7	75	33.3	40	50	58.4	57.1	60	55.6	81	48
Lactobacillus spp.	28	20	33.3	31	25	41	20	26	28	20	8.1	21.4	35	40.4	11	27
Micrococcus spp.	ND	ND	ND	ND	ND	ND	5	40.7	ND	ND	ND	ND	ND	ND	ND	15
Pediococcus spp.	22	ND	ND	ND	ND	ND	ND	ND	22	ND	8.5	ND	ND	ND	ND	ND
Staphylococcus spp.	10	30	16.7	31	12.5	13.3	ND	ND	10	30	25	21.5	5	4	8	10
Mycoflora																
Arthrinium spp	ND	ND	ND	ND	25	16	11.1	7	ND	ND	ND	33.3	ND	ND	28.5	7
Aspergillus spp.	66.7	20	66	27	35	40	20	34	66.7	20	50	33.3	26.5	43	39	50.4
Cladosporium spp.	33.3	50	34	33	25	20	13	11	33.3	50	15	16.7	19	17	9.5	ND
Fusarium spp.	ND	ND	ND	ND	ND	ND	ND	9	ND	ND	ND	ND	ND	ND	ND	ND
Penicillium spp.	ND	ND	ND	ND	ND	ND	9.7	10.3	ND	ND	ND	ND	20	ND	ND	10
Saccharomyces spp.	ND	ND	ND	ND	ND	ND	11.2	14.7	ND	ND	ND	ND	ND	ND	7	**18.7**
Trichoderma spp.	ND	30	ND	40	ND	ND	24	11	ND	30	25	5	4.1	ND	10	**2**
Ulocladium spp.	ND	ND	ND	ND	15	24	11	3	ND	ND	10	10.7	30.4	40	6	**12.2**

Each value represents the mean of triplicate sample analysis of two experimental replications. ND = Not detected.

MATERIALS AND METHODS

Soft drink samples and storage

Two most popular brands of carbonated non-alcoholic soft drinks (330 ml capacity) marketed in Nigeria [(henceforth referred to as brands A (cola base) and B (orange base) [in this paper] were procured soon after production from the bottling company in Port Harcourt, and immediately transported to the laboratory for this work.

Twelve bottles of each brand were subjected to two different marketing conditions often encountered in Nigeria and many developing countries namely, (i) holding at ambient temperature of *ca* 28°C and (ii) holding in open-air (*ca* 34°C) for 21 days. Prior to exposure to retail marketing conditions, 3 samples of each brand were analysed as indicated below for intitial quality attributes. Subsequently, the samples were then analysed weekly for microbiological quality and changes in pH (a critical quality index).

Microbiological evaluation

Samples (3 of each brand) were withdrawn weekly and shaken several times before opening aseptically (i.e. following surface-sterilization using 70% ethanol). Serial dilutions of the samples were made (10^{-1} to 10^{-3}) using physiological saline. Aliquots (0.1 ml) of the dilutions (including undiluted) were spread-plated on plate count agar (PCA; Oxoid) and potato dextrose agar (PDA; Oxoid) for the enumeration of total aerobic bacterial count (TABC) and fungal population. Plates were incubated at 37°C and 28°C for 48h and 4 days respectively and the colonies enumerated while recording the different morphotypes (Harrigan and McCance 1976; Collins and Lyne 1984; Samson and van Reenen-Hoekstra 1988).

Isolation, characterization and identification of the microorganisms

Representative types of colonies were picked from well-isolated plates (25-30 colonies/plate) and purified by streaking on the appropriate medium. The isolates were transferred onto slants and preserved under refrigeration until needed for further characterization.

The isolates were characterized using microscopy (morphology, Gram and spore reactions and motility test) and biochemical/physiological identification based on production of catalase, coagulase, oxidase, indole, methyl-red and Voges-Proskauer reactions, citrate utilization (IMViC), starch hydrolysis, production of hydrogen sulphide (Kliger iron agar) as well as fermentation of sugars (glucose, maltose, mannitol, sucrose and lactose). Identification of the isolates was carried out following comparison with earlier described characteristics (Collins and Lyne 1984, Krieg and Holt 1984; Sneath et al. 1986).

pH and temperature determination of samples

The pH of the samples (3 of each brand) was determined using a referenced glass electrode pH meter (model 3015, Jenway Ltd., England). The temperature of the samples was also monitored by inserting a thermometer through a punctured crown of the bottle. Two independent experiments were carried out during the work.

RESULTS

The microbial composition of the two different brands is presented in Table I. The initial bacterial flora varied with *Pediococcus* spp. occurring in brand A but not in brand B. In contrast, much higher incidence (30%) of *Staphylococcus* spp. was observed in brand B as opposed to 10% incidence in brand A (Table I). The genera of moulds isolated from the two brands also differed with *Aspergillus* and *Cladosporium* occurring as the only mycoflora in brand A but *Trichoderma* in addition to *Aspergillus* and *Cladosporium* occurred in brand B (Table I).

The succession of the flora became more varied as the time of exposure to the two marketing conditions was extended (Table I). *Bacillus* spp. were the most predominant group in both brands but the incidence increased by approximately 88% in brand A samples while a decrease was observed in brand B samples held under the same ambient condition. In contrast, samples held in the open-air (34°C) showed substantial increase of approximately 103% in brand A but tended to remain unchanged or changed slightly at the end of storage in brand B samples subjected to the same condition (Table I). Holding of samples under ambient condition led to initial (day 7) increase in *Lactobacillus* spp. in brand A samples and a decrease thereafter which contrasted with approximately 2-fold increase observed in brand B samples on day 14. On the contrary, samples held in open-air showed initial sharp decrease in brand A samples followed by a dramatic increase in the two brands and finally, a decline (Table I). Incidence of *Micrococcus* spp. was only detected in the samples after 21 days of exposure to the two different conditions but much higher incidence was observed in samples held under ambient condition (Table I). *Pediococcus* spp. were sporadically detected with time of exposure. Incidence of *Staphylococcus* spp. tended to increase in both brands but became non-detectable with time in samples held under ambient condition while low incidence occurred in both brands exposed to 34°C (Table I). The mycoflora exhibited dramatic trends; with *Artinrinium* spp. being undetected within the first 7 days but became detectable thereafter with higher incidences occurring in samples held under open-air (Table I). A decrease in the incidence of *Aspergillus* spp. was observed in brand A samples held at ambient condition but brand B samples showed an increase. A similar trend occurred in samples held under the sun (open-air) but higher incidences were observed (Table I). A gradual decrease in incidence of *Cladosporium* spp. occurred in both brands but were not detected in brand B at the end of storage (Table 1). *Saccharomyces* spp. were not isolated from both brands until 21 day of exposure to the two different marketing conditions with higher incidences occurring in brand B samples.

The time-course changes in total aerobic bacterial counts (TABCs) of the two brands subjected to the marketing conditions are shown in Fig. 1. All samples exhibited low microbial load until

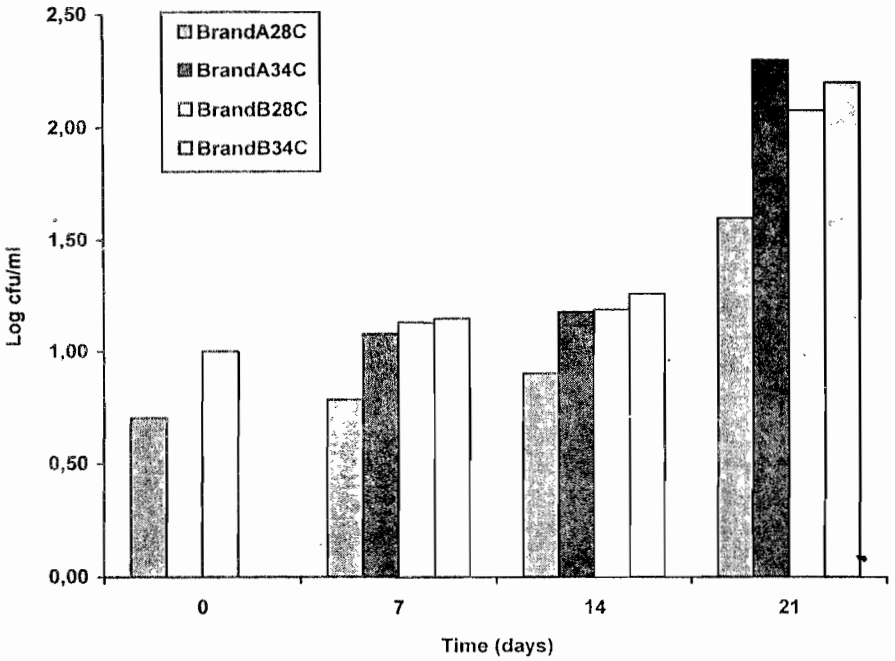

Figure. 1 Time course changes in total aerobic bacterial counts of two brands of soft drinks subjected to two marketing conditions.

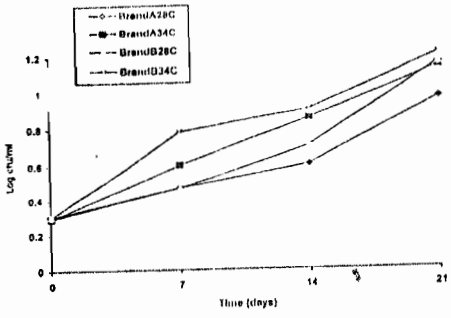

Figure 2. Time course changes in fungal population of two brands of soft drinks subjected to two marketing conditions.

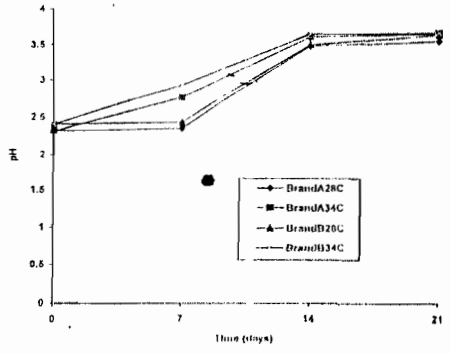

Figure 3 Time course changes in pH of two brands of soft drinks subjected to two marketing conditions.

after 14 days at which point approximately 13- and 9-fold increases were observed in brands A and B samples held in open air (34°C) respectively (Fig. 1). Brand A samples held under ambient condition (*ca* 28°C) showed consistently lower population than those subjected to open-air (34°C).

The fungal population of the samples was generally low ranging between $\log_{10}$ 0.30 and 1.20 cfu ml^{-1}. However, higher populations were observed in samples held in open air with brand B samples showing higher counts (Fig. 2).

The pH increased with storage time in both brands held under the two different conditions but greater increase occurred in samples subjected to open-air (34°C) marketing condition (Fig. 3).

DISCUSSION

The intrinsic characteristi and the processing parameters as well as the distribution practices of a particular food product have considerable impact on the microbial flora and its stability. The heterogeneity of the flora isolated from the soft drinks (Table I) is therefore probably related to a number of factors including poor quality 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 (Sridhar 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 (Panezai 1978).

The differential microbial distribution in the two brands (Table I) may in part be attributed to differences in the chemical composition of the products. It is known that brand A contains caffeic acid, a plant phenolic which has antimicrobial property while brand B does not. However, the dominance of *Bacillus* spp. in both brands 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). On the contrary, the incidence of *Pediococcus* spp. in brand A but not in brand B could be due to the differential pH values (Fig. 2). Similarly, the higher incidence of Staphylococci in brand B (Table I) corroborates the results by Notermans and Heuvelman (1983) which showed greater inhibition of Staphylococci with decreasing pH. The incidence of mixed flora consisting of both thermo-resistant and less thermo-resistant organisms (Table I) has previously been reported in comparable acidic products (Put and De Jong 1982; Vanderzant and Splittstoesser 1992; Efiuvwevwere and Atirike 1998) suggesting under-processing or post-process contamination.

The greater diversity of the mycoflora isolated from brand B (Table I) further suggests the impact of the differential intrinsic properties (formulations) of each product. For example, *Trichoderma* spp. and their characteristic structures; chlamydospores are frequently isolated from citrus fruits and juices (Domsch et al. 1980) thus, presumably, indicating their association with the raw materials of brand B. But the dominance of the two brands by *Aspergillus* spp. and *Cladosporium* spp. is consistent with earlier findings which showed the prevalence of these organisms in raw materials for soft drinks as well as their association with soft drink factory environment (Panezai 1978; Odunfa 1987).

The dynamics of microbial evolution of the different flora as the holding time was extended (Table I), is probably due to induced alteration in product composition. The increase in pH with time (Fig. 2) exemplifies one of such changes which could be attributed to utilization of the preservatives or a deacidification phenomenon previously observed in beverages and acidic products (Vanderzant and Splittstoesser 1992; Buchta et al. 1996; Efiuvwevwere and Akoma 1997). Similarly, yeasts have earlier been implicated as spoilage agents of beverages (Put and De Jong 1982; Thornton and Rodriguez 1996) thus, the present results parrallel such findings since *Saccharomyces* spp. were not detected until after 14 days (Table I); a period which coincided with overt deterioration (i.e. noticeable sedimentation) of some of the products. This therefore establishes some of the complaints by consumers of these products. In addition, it was observed that the colour of the beverages had faded by the 14th day of exposure to the open-air. Perhaps, this was due to photooxidation suggesting loss of potency and efficacy of the preservatives and flavoring agents. It is also known that as temperature increases, less carbon dioxide is dissolved in a liquid medium and this implies less carbonic acid; thereby resulting in the higher pH of samples held under the open-air (Fig 2). These induced changes may partly explain the observed higher microbial (both bacterial and fungal) population in samples subjected to open-air marketing condition (Figures 1 and 2).

This investigation has demonstrated the heterogeneous microbial composition of the most popular soft drinks in Nigeria. But exposure of the products to two common marketing conditions led to remarkable microbial dynamics particularly after 14 days probably due to alteration of the composition and accumulation of metabolic products; resulting in exacerbated microbial quality changes in samples held in open-air (34°C). Microbiologically, these products became sources of potential health risks especially as a result of the high incidences of *Bacillus spp.* and *Aspergillus spp;* both of which contain several pathogenic and mycotoxigenic species. In conclusion, adoption of hazard analysis critical control point (HACCP) system including adequate distribution practices would enhance the microbial quality and stability (i.e. total quality) of these products thereby minimizing their deterioration and economic losses. Until such measures are adopted, it is advised that these products are sold and consumed within 14 days if subjected to the distribution conditions investigated in this work.

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