JJBS

Jordan Journal of Biological Sciences

Volume 4, Number 3, September 2011
ISSN 1995-6673
Pages 157 - 164

Storage Effects on the Quality of Sachet Water Produced within Port Harcourt Metropolis, Nigeria

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Received 17January, 2011; received in revised form 11 May 2011; accepted 12 May 2011

Abstract

The present investigation highlights the effect of storage on the physico-chemical status and bacteriological quality of sachet water produced in Port Harcourt, Nigeria for a period of four months. Ten brands of sachet water were collected within 24 hours of production and stored at ambient temperature. Sub-samples were drawn from the stock samples on monthly basis for physico-chemical measurement and on weekly basis for enumeration of total aerobic heterotrophic bacteria and indicator organisms using ASTM, APHA and WHO analytical methods. pH values increased in all brands to acceptable WHO limits within 8 weeks of storage and gradually decreased toward the end of the experiment. Dissolved oxygen, volatile organic matter and nitrate values decreased throughout the investigation period while phosphate and potassium values increased throughout the investigation period in all brands tested. Total aerobic heterotrophic bacterial count increased gradually in all brands to unacceptable limit within four weeks of storage and gradually diminished to zero level by the end of experiment. Total and faecal coliform appeared in 40% of sachet water samples analyzed within the first three weeks and were no longer detected throughout the investigation period. *Escherichia coli* was isolated in one brand at the onset while faecal *Streptococci* were absent throughout the investigation period. Results of the experiment indicate that 60% of the brands analyzed met the WHO guideline limit for drinking when stored at ambient temperature within four week period. However, storage beyond this period led to diminished aesthetic quality of sachet water and increased proliferation of bacteria to a level deleterious to human health.

Keywords: Sachet water, storage effects, quality, Nigeria.

1. Introduction

Most people living in the major cities of Nigeria do not have access to pipe borne water, probably due to unavailability or inadequacy where obtainable (Omalu *et al*., 2010). People, therefore, resort to the more costly alternative of buying water from vendors; sachet or bottled water became a major source of drinking water.

Sachet water, a brand of packaged water has, therefore, gradually become the most widely consumed liquid for both the rich and the poor in Nigeria. It is the brand of choice to everyone because it is a cheaper alternative to the bottled brand, considered to be the refreshment of the affluent. Hygiene, purity, tastes, and, most importantly, safety is probably amongst various reasons for sachet water consumption. Unfortunately, the problems of its purity and health concerns have begun to manifest (Oladipo *et al*., 2009).

Sachet water is regulated as a food product in Nigeria by National Agency for Foods Drugs Administration and Control (NAFDAC). The agency relies on World Health Organization (WHO) standards for the product regulation, registration and certification. There has been a tremendous improvement in sachet water regulations by NAFDAC as the number of illegal producers has drastically reduced and most brands on sale now have NAFDAC registration.

Sachet water is not completely sterile; it may not be entirely free of all infectious microorganisms. The potential danger associated with sachet water is contamination, which is a factor of the source of the water itself, treatment, packaging materials, dispensing into packaging materials and closure (Omalu *et al*., 2010). Under prolonged storage of packaged water at favorable environmental conditions, total aerobic heterotrophic bacteria can grow to levels that may be harmful to humans (Warburton *et al*., 1992). Total aerobic heterotrophic bacterial counts are sensitive and practical indicators of water treatment efficiency as well as after-growth and biofilm formation. Some of the total aerobic heterotrophic bacteria have been identified as opportunistic pathogens (Rusin *et al*., 1997). These microorganisms can be found in source waters and in treated drinking water. Thus, consumption of water containing large numbers of total aerobic heterotrophic bacteria can lead to diseases such as gastroenteritis and mucous membrane infections particularly in persons whose immune systems are

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compromised by AIDS, organ transplantation or chemotherapy (Grabow, 1996; Rusin *et al*., 1997).

The objective of this study, therefore, is to examine the effect of prolonged storage on the physico-chemical status and bacteriological quality of sachet water that has been hitherto certified for consumption by appropriate authority.

2. Materials and Methods

Ten brands of sachet water with NAFDAC certification were randomly collected in different parts of Port Harcourt metropolis, Nigeria in bags within 24hours of production and stored in a room at ambient temperature. Sub-samples were drawn from the stock samples in triplicates for physico-chemical characterization and bacteriological assay using ASTM, APHA and EPA analytical methods (EPA, 1996; APHA, 1998; ASTM, 1999).

Physico-chemical parameters of the sachet water were analyzed immediately after collection and subsequently thereafter on a monthly basis. pH, temperature and dissolved oxygen of the sachet water were determined electrometrically with a multi-parameter data logger (Hanna model HI991300, Hanna Instruments, Singapore). The meter was calibrated prior to use with 0.01N and 0.1N standard potassium chloride solutions (according to the manufacturer's specifications), and buffer standards (obtained from AccuStandard Europe) of pH 4 and 7 at room temperature. Volatile organic matter was determined by weight lost on ignition method at 550±50^{o}C in accordance with APHA 2540E. Nitrate and phosphate in sachet water samples were determined colorimetrically by UV/Visible spectrophotometer in accordance with EPA 352.1 and APHA 4500. Potassium was analyzed with Flame Atomic Absorption Spectrophotometer (FAAS) in accordance with APHA 20th edition 3111B. Samples were analyzed by direct aspiration in an acidic medium into an Air/Acetylene flame at specified wavelength for potassium.

Bacteriological analysis of the sachet water was carried out immediately after collection and subsequently thereafter on weekly basis. Total aerobic heterotrophic bacterial count was determined following the heterotrophic plate count method, using spread plate technique in accordance with ASTM D5465-93 and APHA 9215. 1ml of the sample or 0.1ml of final dilution of the sample in sterile Ringer's solution where necessary was aseptically introduced onto dry nutrient agar surface in triplicates spread plated with a glass spreader and incubated in an inverted position at 35±2^{o}C for 18-24 hours. Plates containing 30 - 300 were counted at the end of the incubation period. Total coliform, faecal coliform, *Escherichia coli* and faecal *Streptococci* were determined by using Membrane Filtration Technique in accordance with ASTM D5392-93, APHA 9222B and WHO Guidelines for Drinking Water Quality (2001, Volume 3). Filtration unit comprising of Erlenmeyer flask, vacuum source and porous support were assembled and with the aid of a flame sterilized forceps, a sterile membrane filter (0.45µm Millipore) was placed on the porous support. The upper funnel was placed in position and secured with appropriate clamps. 100ml of sachet water sample was aseptically poured into the upper funnel and suction applied to create a vacuum. After the sample was passed through the membrane filter, the filtration unit was taken apart and with the aid of a sterile forceps. The membrane filter was placed in the Perti dish on the pad that had been saturated with McConkey broth for total and faecal coliforms, Eosine Methylene Blue agar for *Escherichia coli* as well as Slanetz and Bartley agar for faecal *Streptococci*. The upper funnel was then removed and rinsed with 200ml of sterile Ringer's solution prior to use for the next sample. All plates were incubated in inverted position at 37±2^{o}C (total coliforms) and 44±2^{o}C (faecal coliforms, *Escherichia coli* and faecal *Streptococci*) for 18-24 hours.

3. Results and Discussion

The pH value of the sachet water samples is presented in Fig. 1. The pH values varied from 4.43 to 7.71 averaging 6.07 throughout the investigation period. pH was outside WHO limits in all the 10 samples analyzed at the onset of the investigation (Week 0) while an increase in pH was observed in all the samples up to week 8 with about 40 to 100% falling within WHO limit followed by a decline between Week 8 and Week 16.

pH is one of the parameters that addresses the aesthetic quality of water such as taste which has no serious health significance (WHO, 1996). However, pH played a significant role in determining the bacterial population growth and diversity in sachet water. Increase, observed in pH, could be attributed to the production of basic metabolic waste products by increasing bacterial population. In their review, Prescott *et al.* (1999) stated that microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products.

The temperature readings of the sachet water samples are as presented in Table 1. The temperature values ranged from 27.1 to 28.5^{o}C averaging 27.9^{o}C. The temperature values obtained throughout the investigation period fall within the optimal growth range for mesophilic bacteria including human pathogens. Prescott *et al.* (1999) reported 20-45^{o}C as optimal growth temperature for mesophilic microorganisms. According to WHO report (1996), the microbiological characteristics of drinking water are related to temperature through its effects on water-treatment processes and its effects on both growth and survival of microorganisms. Consequently, growth of nuisance microorganisms is enhanced by warm water conditions and could lead to the development of unpleasant tastes and odors.

The dissolved oxygen values obtained for the sachet water samples are as presented in Fig. 2. The dissolved oxygen values ranged from 4.10-7.85 mg/l averaging 4.92 mg/l. Growth of aerobic and facultative anaerobic bacteria will be enhanced by the presence of dissolved oxygen in sachet water. A decrease in dissolved oxygen was generally observed in all sachet water samples throughout the investigation period; an indication of possible bacterial respiration of organic materials by the bacterial flora of the sachet water samples tested. WHO (1996) reported that there is tendency for the level of dissolved oxygen to fall with time indicating possible microbial respiration of organic materials amongst other reasons.

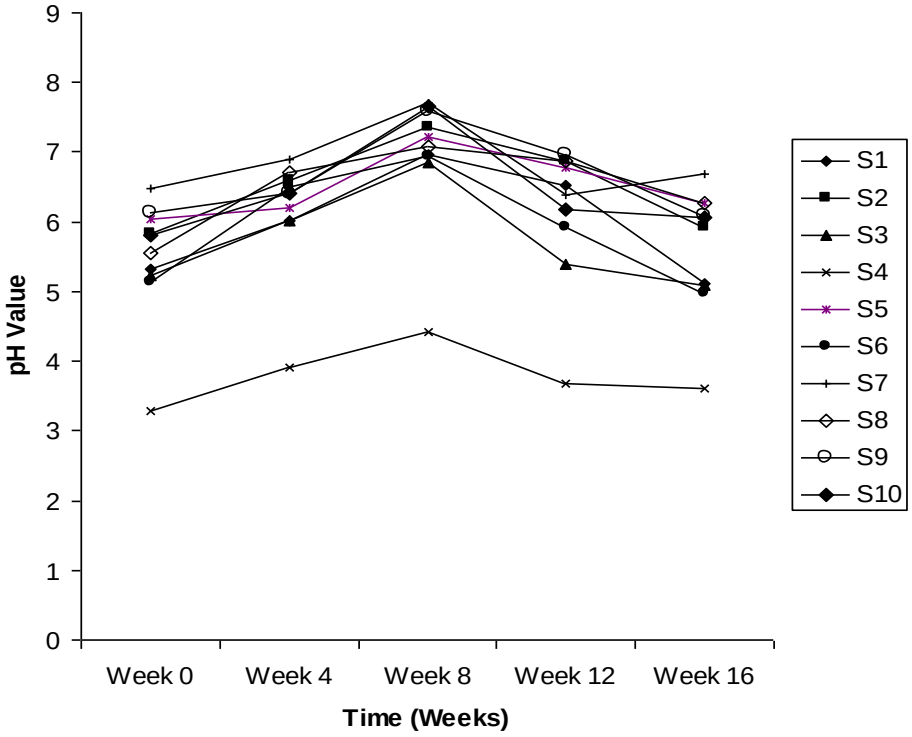

Figure 1. pH Values of Sachet Water in 16 Week Period.

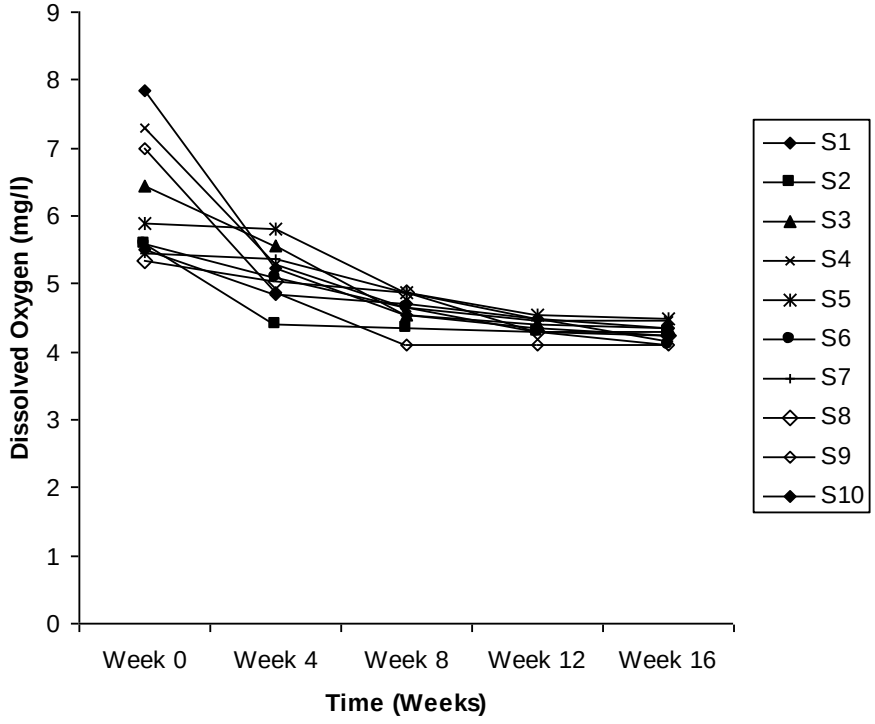

Figure 2. Dissolved Oxygen Values of Sachet Water in 16 Week Period.

Table 1. Temperature (^oC) of Sachet Water in 16 Week Period

Sample code / Period	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Week 0	27.7	27.5	27.1	27.7	27.6	27.3	27.3	27.1	27.1	27.6
Week 4	27.8	27.5	27.7	28.0	27.6	27.8	27.6	27.5	27.6	27.5
Week 8	28.3	28.3	28.3	28.1	28.2	28.3	28.3	28.3	28.4	28.3
Week 12	27.6	27.5	27.3	27.6	27.3	28.0	28.0	27.6	27.6	27.6
Week 16	28.4	28.3	28.3	28.3	28.4	28.5	28.3	28.3	28.3	28.4

Volatile organic matter values obtained for the sachet water samples are as presented in Fig. 3. The values of volatile organic matter ranged from 1.0-2.5 mg/l. Volatile organic matter values decreased throughout the investigation period. Decrease in volatile organic matter could be attributed to their uptake as substrate for energy production and growth (Pelczar *et al*., 1993).

Nitrate values obtained for the sachet water samples are as presented in Fig. 4. The values of nitrate ranged from 0.54-1.09 mg/l. Nitrate values decreased throughout the investigation period. Decrease in nitrate values could be attributed to their utilization by microorganisms for growth and reproduction (Prescott *et al*., 1999)

Phosphate and potassium values are also presented in Fig. 5 and 6, respectively. The values of phosphate and potassium ranged from 0.62-3.48 mg/l and 0.14-2.98 mg/l, respectively. Phosphate and potassium values increased throughout the investigation period. This could be attributed to microbial death and accumulation of metabolic waste (Prescott *et al*., 1999).

Total aerobic heterotrophic bacterial counts obtained from sachet water samples are as presented in Table 2. The counts of total aerobic heterotrophic bacteria ranged from 0.0 to 2.7×10^4 cfu/ml of the sachet water samples. This is in agreement with Olaoye and Onilude (2009) who observed that varying levels of microbial contamination were recorded in samples from the different sampling locations. A gradual increase in total aerobic heterotrophic bacterial counts was observed in all the brands tested up to week 8 followed by decease in counts up to the end of the experiment, a growth pattern typical of microorganisms growing in closed system (Brock and Madigan, 1988).

The total aerobic heterotrophic bacterial count method determines the general microbiological quality of treated drinking water (Allen *et al*., 2002) WHO drinking water quality specifications allow total aerobic heterotrophic bacterial counts of 100 cfu/ml (Allen *et al*., 2002). However, this limit was exceeded by all the sachet water samples tested after four weeks of storage.

The result of total aerobic heterotrophic bacterial count obtained in this study is in agreement with the findings of Warburton *et al*. (1992). In a study involving the determination of the microbiological safety of bottled water stored for 30 days, Warburton *et al*. (1992) reported that the total aerobic heterotrophic bacterial counts increased considerably to a level detrimental to human health when the water was stored at room temperature.

Total coliform bacteria (Table 2) were detected in 40% of the brands of sachet water analyzed within the first three weeks while faecal coliform were detected in 10% of the brands of sachet water analyzed at the onset of the investigation. However, they were not detected after this period. Indicator organisms loose viability in freshwater environment with time (WHO, 2001).

Total coliforms are widely used as indicators of the general sanitary quality of treated drinking water while faecal coliforms give a much closer indication of faecal pollution (Ashbolt *et al*, 2001). WHO limit is that none should be detected. Unlike total aerobic heterotrophic bacteria, total and faecal coliform counts did not increase in sachet water samples tested. Among the criteria for indicator organisms, Prescott *et al*. (1999) stated that indicator bacterium should not reproduce in the contaminated water and produce an inflated value. This justified the choice of coliform bacteria by WHO as indicator organisms (WHO, 2001).

The counts of faecal *Streptococci* (Table 2) remained 0.0 cfu/ml in all brands tested throughout the investigation period while *Escherichia coli* was detected in one brand at the onset of the investigation (Week 0). The presence of faecal S*treptococci* in potable water is an additional or a secondary indicator of faecal pollution while the presence of *Escherichia coli* confirms faecal pollution of potable water, which is not acceptable (Ashbolt *et al*, 2001; WHO, 1996). Detection of *Escherichia coli* in one brand therefore is a confirmation of its faecal contamination.

4. Conclusion

The objective of this work was to evaluate the effect of prolonged storage on the physico-chemical status and bacteriological quality of sachet water samples collected within Port Harcourt metropolis, Nigeria.

The analytical results revealed that prolonged storage caused an increase in pH up to week 8 followed by a decrease up to the end of the experiment in all pure water samples tested. The presence of dissolved oxygen coupled with availability of organic material and nutrients aided continuous and rapid proliferation of bacteria in sachet water tested over time.

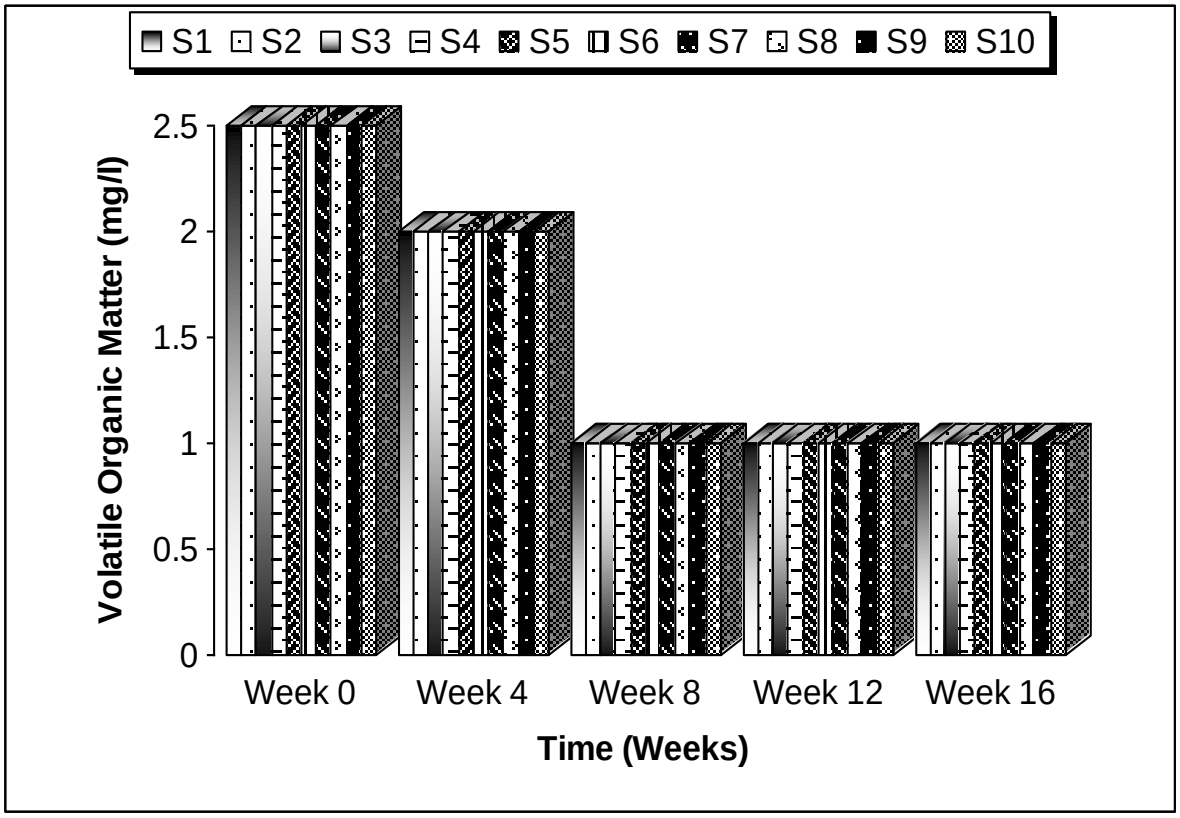

Figure 3. Volatile Organic Matter Values of Sachet Water in 16 Week Period.

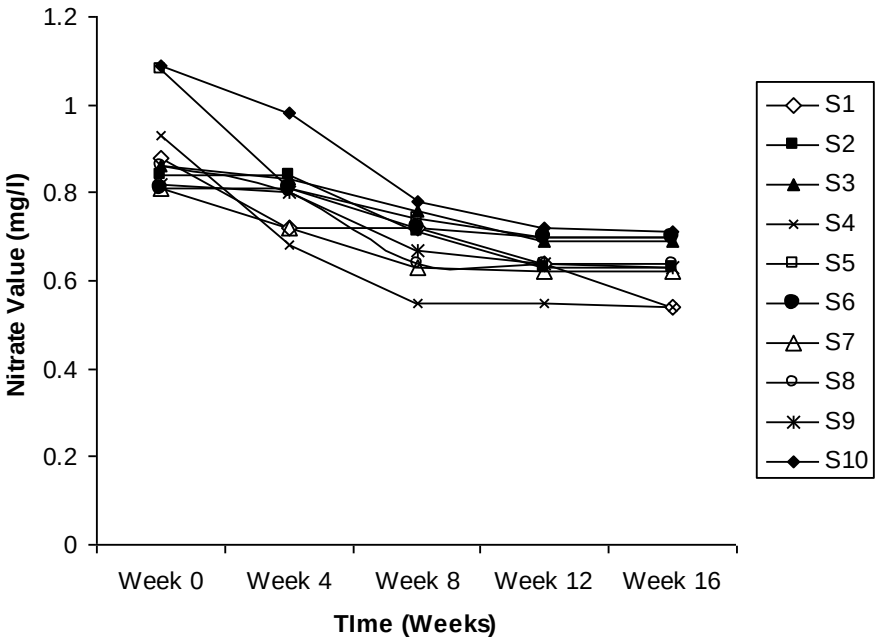

Figure 4. Nitrate Values of Sachet Water in 16 Week Period.

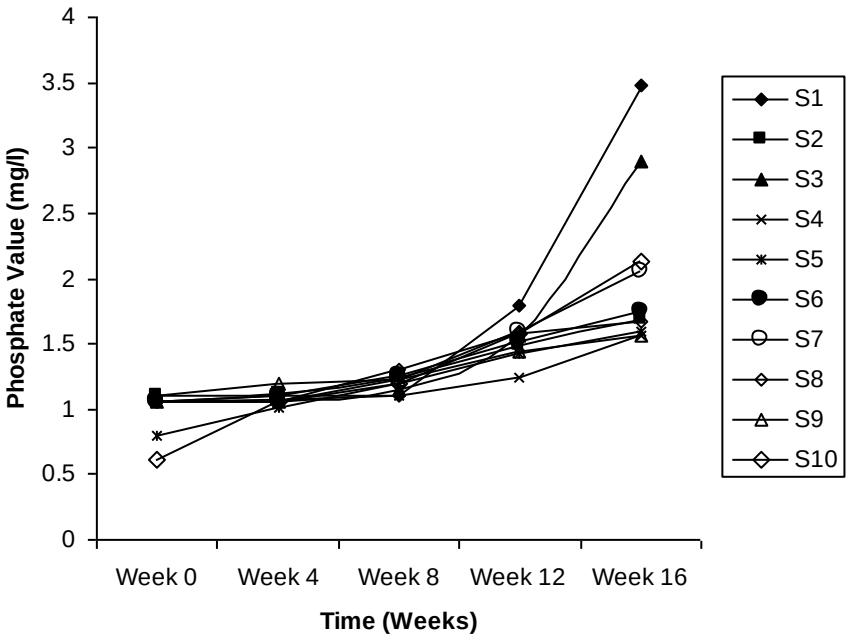

Figure 5. Phosphate Values of Sachet Water in 16 Week Period.

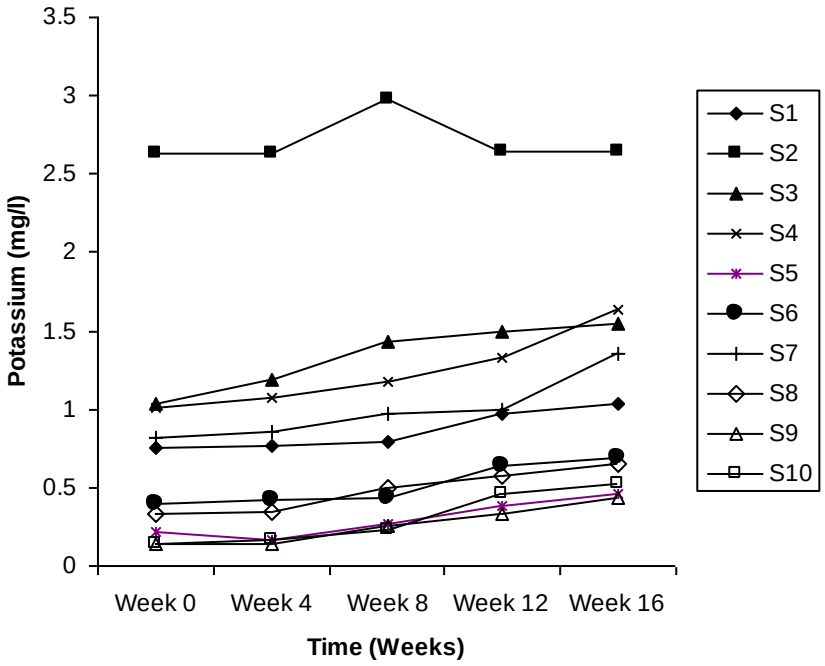

Figure 6. Potassium Values of Sachet Water in 16 Week Period.

Table 2. Total Aerobic Heterotrophic and Pathogenic Bacterial Count (cfu/ml)

Period	Bacteria Type	Sample Code S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Wk 0	THB	1.0	37.0	12.0	4.0	1.0	1.0	1.0	4.0	2.0	2.0
	TC	0.0	4.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
	FC/EC	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	FS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 1	THB	5.0	40.0	48.0	12.0	6.0	1.0	6.0	14.0	2.0	11.0
	TC	0.0	2.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0
	FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 2	THB	10.0	46.0	54.0	22.0	65.0	2.0	25.0	32.0	3.0	12.0
	TC	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 3	THB	13.0	97.0	94.0	53.0	71.0	5.0	28.0	42.0	5.0	22.0
	TC	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 4	THB	23.0	127.0	97.0	72.0	88.0	7.0	40.0	50.0	12.0	5.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 5	THB	3.9×10^3	146.0	4.9×10^3	1.0×10^3	1.0×10^3	1.3×10^2	3.0×10^3	4.0×10^2	1.5×10^3	1.8×10^3
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 6	THB	5.4×10^3	1.4×10^4	6.6×10^3	3.4×10^3	1.4×10^3	2.6×10^2	4.1×10^3	1.2×10^3	2.8×10^3	1.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 7	THB	8.4×10^3	2.7×10^4	4.0×10^2	7.3×10^2	2.8×10^3	4.0×10^2	5.4×10^3	3.2×10^2	5.6×10^2	1.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 8	THB	2.5×10^3	6.0×10^3	1.7×10^2	1.2×10^2	4.0×10^2	1.9×10^3	9.7×10^2	1.4×10^2	2.1×10^2	1.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 9	THB	2.5×10^3	1.2×10^3	96.0	1.2×10^2	1.0×10^2	2.0×10^3	9.6×10^2	52.0	1.7×10^2	1.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 10	THB	2.5×10^3	1.1×10^3	82.0	1.2×10^2	26.0	122.0	1.1×10^2	21.0	35.0	1.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 11	THB	1.6×10^3	3.6×10^2	80.0	56.0	4.0	42.0	56.0	20.0	33.0	1.0×10^2
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 12	THB	1.1×10^3	12.0	20.0	11.0	1.0	42.0	11.0	20.0	21.0	2.0
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 13	THB	16.0	12.0	1.0	2.0	1.0	2.0	1.0	2.0	2.0	1.0
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 14	THB	3.0	1.0	1.0	2.0	0.0	2.0	0.0	1.0	1.0	1.0
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 15	THB	2.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wk 16	THB	2.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
	TC/FC/FS/EC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

THB: Total heterotrophic bacteria, TC: Total coliform, Faecal colifirm, FS: Faecal *Streptococci*, EC: *Escherichia coli*

All pure water samples analyzed exceeded WHO limit of 100 cfu/ml for total aerobic heterotrophic bacteria within weeks 4 and 8. Total and faecal coliform appeared in 40% of sachet water samples analyzed within first three weeks and died off. They lost viability in freshwater environment with time. No faecal *Streptococci* was detected throughout the investigation period while *Escherichia coli* was detected in one brand.

The present study has revealed that sachet water when stored at room temperature for a long period can increase total aerobic heterotrophic bacteria to a level that may be harmful to human health.

5. Recommendations

Expiry date of sachet water produced in Nigeria should not exceed four weeks from the date of production. The public should be sensitized not to drink sachet water that has exceeded four weeks from the date of manufacture. The regulatory body should promulgate standardized method of storage of sachet water in order to increase its shelf life. Periodic sanitary inspection of sachet water factories by the regulatory body is absolutely necessary to ensure conformity.

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