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Effects of storage on some properties of selected soft drinks sold in Nigeria

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Abstract

This study was aimed at investigating the effect of storage on some properties of selected soft drinks sold in Nigeria.Two different brands of PET bottled carbonated soft drinks were analyzed for the effect of storage on the pH, titrable acidity, specific gravity, total soluble solids content(TSSC) and antimony leaching using standard methods. Five bottles of the samples were kept at room temperature while five samples were exposed to sunlight. The drinks were analyzed after 3, 6, 9, 12 and 15 weeks of storage using standard methods. One bottle of each brand was analyzed immediately before storage and they served as control. The results of the analyses show that the pH of the samples decreased with time, the TSSC, the specific gravity, the total acidity and the rate of antimony leaching of the samples increased with storage time. Under sunlight condition, the antimony concentration for sample 1 after 15 weeks of storage increased from 0.002-0.015ppm, while that of sample 2 increased from 0.001-0.007ppm. However, for the samples that were not exposed to sunlight, the antimony concentration for sample 1 increased from 0.003-0.011ppm, while the antimony concentration in sample 2 increased from 0.001-0.003ppm, which is below WHO permissible limit of 0.006ppm. For the samples exposed to sunlight, the acidity of sample 1 increased from 0.008 – 0.032% while that of sample 2 increased from 0.025 – 0.034% and the acidity for the samples stored at room temperature increased from 0.008 -0.194% and 0.025 -0.031 for samples 1 and 2 respectively. At room temperature condition, the TSSC was found in sample 1 to be 14.96% and 0.248 in sample 2 at the 15th week. For the samples exposed to sunlight, the TSSC was found to increase from 12.37 – 14.96 and 0.000 – 0.260 in sample 1 and sample 2 respectively at the 15th week. The Specific gravity for exposed samples at the 15th week increased from 0.9921- 1.0012 in sample 1 and 0.9999 – 1.0014 in sample 2 while for the room temperature samples, it increased from 0.9921 – 1.0010 in sample 1 and 0.9999 – 1.0013 in sample 2. The highest decrease in pH was found in sample 2 (4.06 – 3.98) at the both storage conditions.

Introduction

Soft drinks are sold in cheap plastic or glass bottles and also in cans. The packaging materials can introduce contaminants if not adequately rinsed before use or handled before consumption. Plastic bottles made from poly(ethylene terephthalate) (PET) are increasingly used for beverages such as soft drinks, mineral water, beer, among others, most of the PET resins are sold as food grade material for beverage packaging. In comparison to other packaging plastics, PET bottle is one of the most inert polymers with good barrier properties against moisture, oxygen and CO_2 and with a very low migration tendency of its constituents [1]. However, migration of substances from PET bottles into beverages is not completely negligible. Polymer additives, catalyst residence, degradation products, polymerization side-product or residual monomers are potential migrants [1].

PET is polyester of terephthalic acid and ethylene glycol. 90% of the PET bottles manufactured worldwide employs Sb_2O_3 (antimony trioxide) as a catalyst. Soft drinks in PET bottles contain several hundreds of mg/LSb, with much of this Sb apparently due to leaching from PET bottle [2].

The contents of the PET bottle and the temperature, at which it is stored, both appear to influence the rate and magnitude of leaching of organic and inorganic compounds from PET bottle. The storage time, concentration of migrant in the polymer, type and nature of migrant and its solubility in food also influence the mass transfer of these potential migrants from PET bottle to its content[1].

Most times, people expose these beverages bottled in PET to intense sunlight in the course of transporting them from the production point to the selling point. Sometimes, they are also left under sunlight in the markets. Some people even leave some of these beverages in their cars for a long time before consumption. Some are consumed just few days to their expiry date. Therefore, it is very important to understand whether exposure to sunlight may affect the quality of these beverages in PET bottles. It is also important to understand if storage time and storage temperature can affect the content of these beverages in PET bottles.

Antimony trioxide (Sb_2O_3) is a suspected carcinogen and is listed as a priority pollutant /migrant from PET bottle into its content by the USEPA, the EU and the German Research Foundation [1].

Materials and methods

Sample collection

Two different brands of PET bottled soft drinks (all colored) sold in Nigeria were purchased from ninth mile area of Enugu State. The carbonated soft drinks were altogether twenty-two samples. Each brand name, batch number, manufacturing and expiry dates are given Table

Table 1: carbonated soft drinks used.

Samples	Batch number	Manufacturing date	Expiry date	Number of drinks purchased
1 (Cocacola)	A0601:4156	22/02/13	21/08/13	11
2 (Lacasera Apple)	5220412640L4	12/04/13	11/10/13	11

Sample preparation

Before storage, one bottle each of the two different brands of the carbonated soft drinks was analyzed to serve as controls.

Ten (10) bottles (i.e. five bottle of each brand of the beverages) were kept at room temperature and the following tests were carried out; antimony leaching test, change in pH, TSSC test, change in specific gravity and titrable acidity after 3 weeks, 6weeks, 9 weeks, 12 weeks and 15 weeks of storing the drinks in room temperature.

The remaining ten bottles (i.e. five bottles of each brand) were exposed to sunlight and the following test were carried out; change in pH, change in specific gravity, total dissolved solid(TSSC), titrable acidity and antimony leaching test after 3 weeks, 6 weeks, 9 weeks, 12weeks and 15 weeks of exposure.

Leaching test

A portion (100ml) of the beverage was boiled on a hot plate till the volume reduced to about 30ml. 10ml of per chloric acid and 10ml of HNO_3 were added to the solution. It was boiled for 10mins after which 20ml of H_2O_2 was added to it. The solution was further boiled until it becomes colourless and reduced to 40ml, then it was cooled and 5ml of saturated boric acid solution was added to it and filtered with no12cm Whatmann filter paper. The filtrate was received in a 50ml volumetric flask and subsequently made up to mark with de ionzed water and labeled accordingly for AAS analysis. The results of the samples exposed to sunlight at varying days were compared with the ones kept at room temperature.

pH

The pH of the samples were measured using a pH metre.

Titrable Acidity

A 10ml of diluted product was titrated (10% solution of the sample) with 0.1M KOH solution until the substance reached a pH value between 8.2-8.4, corresponding to the end point of phenolphthalein (AOAC, 1995). Readings were taken with a pH meter (Techmel and Techmel). When this value was reached, the spent KOH was noted and the acidic percentage of the substance was calculated using the following equation, with the result being expressed as percentage of citric acid.

$$\text{Acidity (\% citric acid)} = \frac{100 \times Nap \times V \times F \times Meg}{volume\ of\ sample}$$

Where V	=	Volume of KOH
Nap	=	Normal Concentration of KOH (0.I N)
F	=	Normality Correction Factor (0.007)
Meg/g	=	Miliequivalent per gram of Citric Acid (23.35)
Sample	=	Volume of sample taken (10ml)

Specific Gravity (Amerine and Oguh, 1980)

The cleaned density bottle was dried in oven at 105°C, cooled inside desiccator and weighed to constant weight. It was filled with distilled water and weighed again. The distilled water was poured away and the bottle rinsed with ethanol. The bottle was filled with the sample and the weight taken. The specific gravity of the sample was calculated thus;

$$\text{Specific Gravity} = \frac{mass\ of\ water}{mass\ of\ distilled\ water}$$

Determination of sugar or TSSC

The TSSC was determined using refractometry. Results were reported in °Brix or g/100ml.

The Brix scale or °Brix is numerically equal to the percentage of sugar and other solids dissolved in the solution. The food industry uses this scale for measuring the approximate amount of sugars in fruit juices and other beverages. Thus a solution that is 25°Brix has 25g of sugar per 100 of solution (Ball, 2006).

Risk Assessment/ calculation

In order to estimate the risk caused by long time exposure to Sb, chronic daily intake (CDI) was calculated.

CDI - chronic daily intake

$$CDI_{injection=} \frac{Cs \times IRS \times Ef \times ED \times CF}{BW \times AT}$$

Cs = Exposure point concentration (USEPA, 2011)

IRS = ingestion rate: $100mg.d^{-1}$(USEPA, 2011)

Ef = exposure frequency: 350d//a (USEPA, 2011)

ED = exposure Duration: 30a (USEPA, 2011)

BW = Body weight: 70kg (USEPA, 1991)

aAT = averaging time for carcinogens = 365 x 70d (USEPA, 2002)

bAT = averaging time for non-carcinogens = 365 x EDd (USEPA, 2002)

Cf = unit conversion factor: 10^{-6} kg mg^{-1}(USEPA, 2002)

Cancer risk = CDI x Sf

Total cancer risk = $\sum^{n}_{k=1} CDI_k \times SF_k$

$$SF = \frac{1}{6}$$

Results and discussion

The results of antimony concentration of the samples

The results of antimony concentration in samples 1 and 2 are shown in figure 1a and 1b,

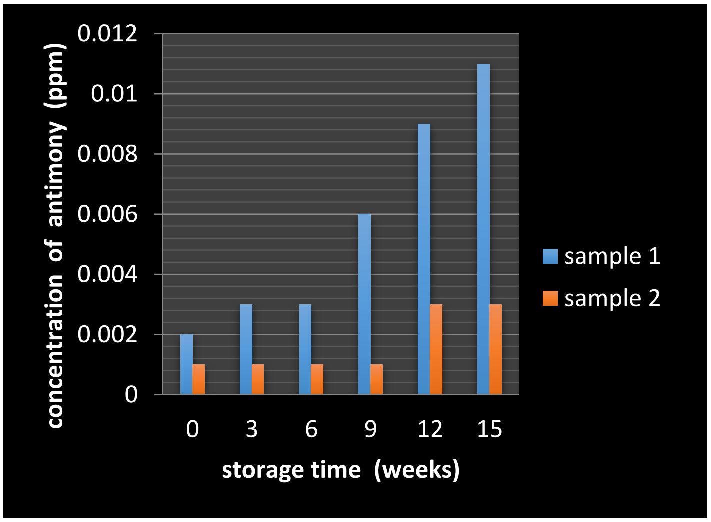

FIG.1A. Concentration of antimony on samples stored at room temperature.

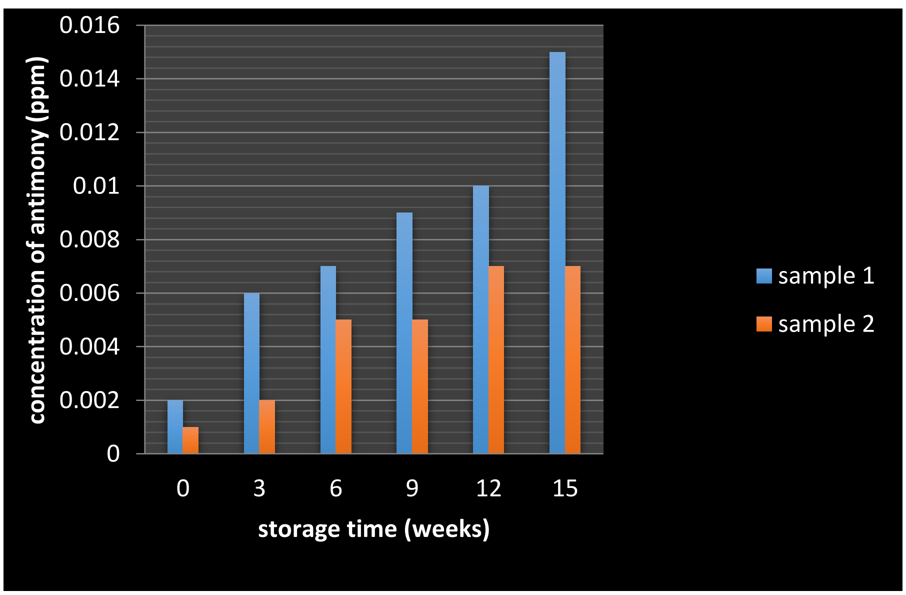

FIG. 1B.Concentration of antimony on samples exposed to sunlight.

The result of antimony concentration for samples 1 and 2 are shown in figures 1a and 1b

Before storage, the antimony concentration for samples 1 and 2 were 0.002ppm and 0.001 which is far below the WHO permissible limit of 0.006ppm.the result showed increase in antimony with increase in storage time. For sample 1, the concentration of sample exposed for 6 weeks exceeded WHO permissible limit.

For sample 2, the concentration of the sample exposed for 12 weeks (0.007ppm) exceeded the WHO permissible limit. The highest concentration of antimony for sample 1 stored at room temperature and exposed to sunlight at 115th week were 0.011ppm and 0.015ppm respectively while for sample 2, stored at room temperature and exposed to sunlight were 0.003 and 0.007 respectively. The higher migration level observed for the sunlight exposed samples may be due to the degradation of PET bottle, activation energy and diffusion coefficient of antimony(Takahashi et al., 2008). Sample 2 showed that at the 12th week, the rate of antimony leaching remained constant in both storage conditions. This observation was in line with the findings of Ying- yang et al.,(2014) who observed an increase in antimony leaching upto the 14th week of storage but the releasing rate decreased with storage time indicating that Sb released can become stable under long term storage. It is also in line with findings of keresztes et al., (2009) that investigated 10 different Hungarian water and observed that antimony leaching increases rapidly during that first storage period until its diffusion reaches a steady state.

Titrable acidity

Effect of storage on the titrable acidity of the samples: Figures 2a and 2b showed the effect of titrable acidity for the samples at both storage Conditions.

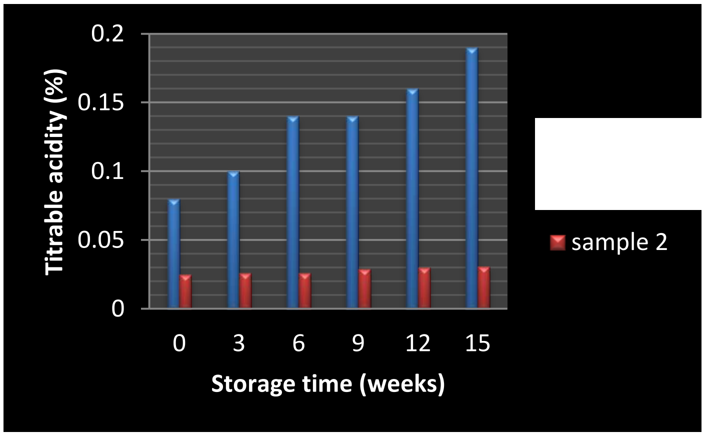

FIG.2A. Change in titrable acidity for samples stored at room temperature.

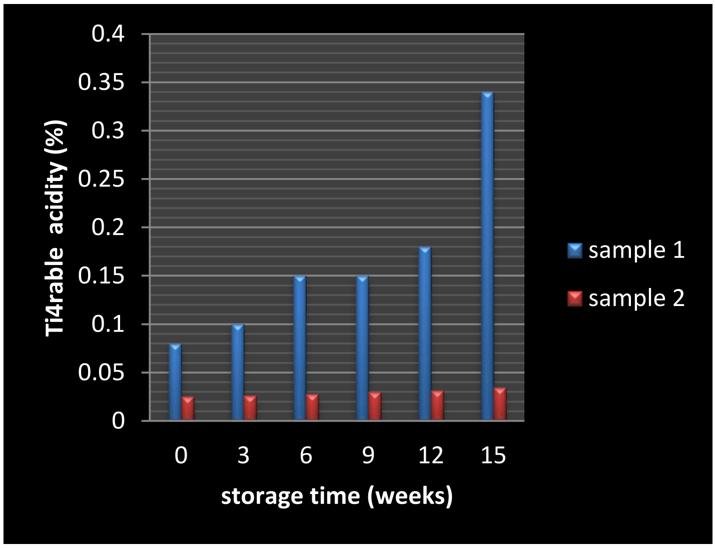

FIG.2B. Change in titrable acidity for samples exposed to sunlight.

Acidity increased with increase in storage time. The percentage acidity value for sample 1 and 2 were 0.08% and 0.025% before storage and showed that same value at both storage conditions. For the control sample and after 3 weeks of storage, sample 1 showed uniform value at 6 and 9 weeks with storage under sunlight showing higher values. The increase in acidity maybe due to the formation of organic acid by ascorbic acid present in juice. The results are in agreement with the findings reported by [4].It is clear that temperature accelerated the biochemical reaction that took place in soft drinks and increased the acidity of the samples. The highest values for acidity for both samples were observed at the 15th week for the samples under different storage condition. The observation is inline with the findings of [5]in the study of effect of sugar levels and different storage conditions on the quality of banana ready-to-serve-beverage (RTS). The samples under sunlight showed high increase in acidity with time than those stored at room temperature. The increase in titrable acidity at both storage conditions may be due to carbon dioxide, citric acid and other regulators purposely added to the soft drinks during preparation to improve taste or act as a preservative. This study was in line with [6]who observed an increase in titrable acidity of banana and sapota beverage stored at ambient temperature (35^0c – 36^0c).

pH

Effect of pH of the samples at different storage conditions.

Figures 3a and 3b show the pH for sample at different storage conditions. The pH decreased with increase in storage periods for the samples at different storage conditions. The pH of samples 1 and 2 before storage were 4.80 and 4.06 and at 3 weeks, the pH became 4.81 and 4.04 respectively.

At 15th week, the pH for the two samples were 4.78 and 3.98 respectively showing a decrease with increase in storage period irrespective of the storage conditions.

This observation is in line with the findings of Yadav et al., (2013) as they study the effects of sugar levels and different storage conditions on quality of banana RTS beverage. Similar trends were reported by [6]in case of banana and sapota beverage stored at different temperatures for 180 days.

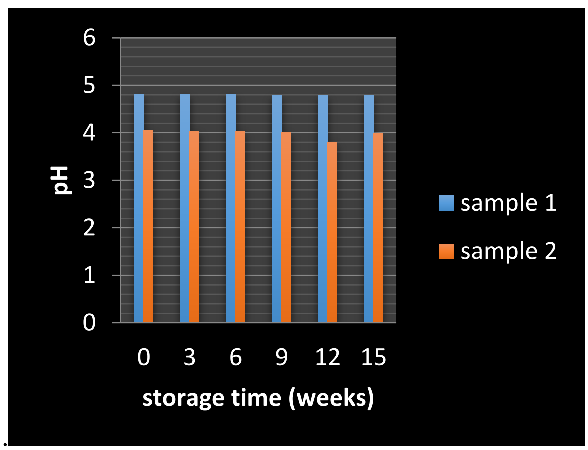

FIG.3A. Change in pH for samples stored at room temperature.

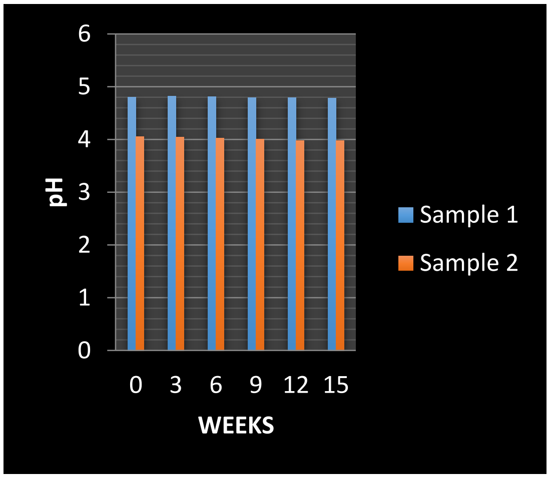

FIG.3B. Change in pH for samples exposed to sunlight.

Total Soluble Solid Content

The result of the effect of storage conditions on the total soluble solid content (TSSC) for the samples are shown in Fig 4a and Fig 4b.

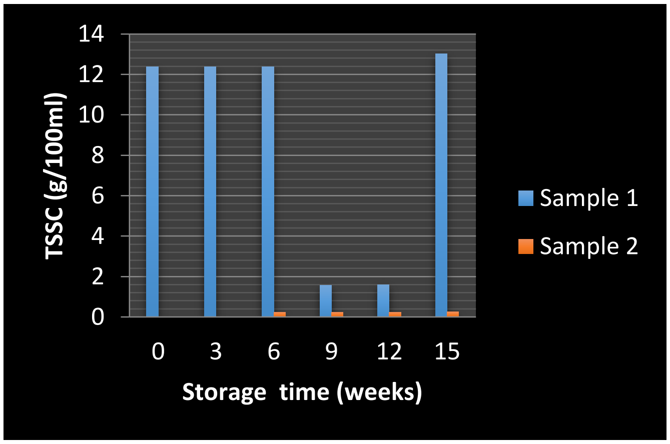

FIG.4A. Change in TSSC for samples stored at room temperature.

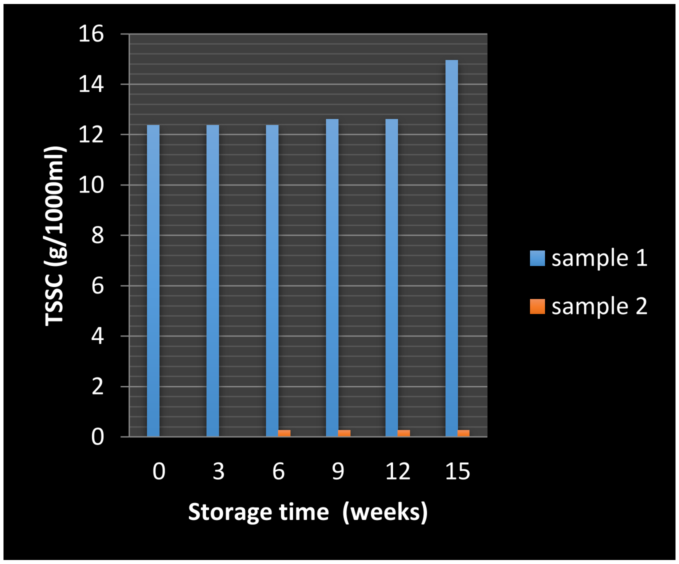

FIG.4B. Change in TSSC for samples exposed to sunlight.

The total soluble solid content for sample 1 at 0 week, 3 weeks and 6 weeks were 12.37 brix^0 both at room temperature and under sunlight. TSSC for sample 2 at 0 week and 3rd week were the same 0.00 at both room temperature and under sunlight. On the 9th week, at both storage conditions, sample 1 showed1.56 brix^{0}and 12.61brix^0 at room temperature and under sunlight respectively while sample 2 showed 0.244 and 0.260brix^0 respectively. The result revealed that the TSSC increased rapidly when stored under sunlight than at room temperature which is in line with the findings of [7] in qualitative evaluation of mixed fruit based ready to serve (RTS) beverage.

Effect of storage condition on the Specific Gravity of the samples

The effect of storage conditions on the specific gravity of sample 1 and 2are shown in Fig 5a and 5b.

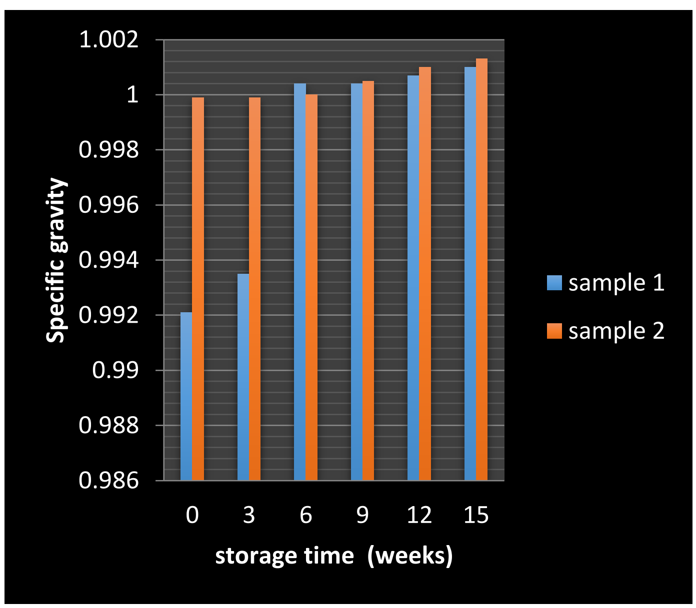

FIG.5A. Change in specific gravity for samples stored at room temperature.

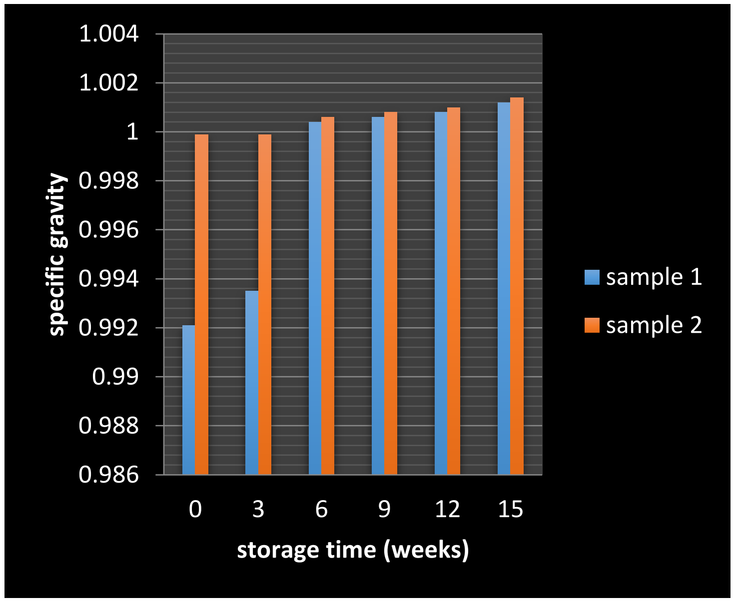

FIG.5B. Change in specific gravity for samples exposed to sunlight.

Before storage, the specific gravity for sample1 and sample 2 were 0.9921 and 0.999 respectively. The specific gravity increased with increase in storage time at both storage conditions. The samples exposed to sunlight increased more rapidly than unexposed samples. Sample 1 showed that the specific gravity for the unexposed samples and exposed samples were 1.0010 and 1.0012 respectively. The observed increase in specific gravity of the samples may be as a result of the activities of sugar which is directly evidenced by the increase in TSSC contents with storage time. The results of the effects of storage on specific gravity of soft drinks are in agreement with works of [8].

Cancer risk calculation

The chronic daily intake and cancer risk for the analyzed samples were calculated based on Sb released from PET bottles stored at room temperature and under sunlight are shown

Table 2. The CDI and the calculated cancer risk of antimony.

S/No	Sample	Exposure Duration	Sb					
			Conc at room temp. storage (ppm)	Conc on Exposure to sunlight(ppm)	$CDI_{injection}$(roomtemp)== CsxIRSxEfxED BW x ATx CF	$CDI_{injection}$(sunlight) = CsxIRSxEfxED BW x AT x CF	Cancer risk @ room temp= CDI x Sf	Cancer risk @ sunlight exposure= CDI x Sf
1	1	0	0.002	0.002	1.17×10^{-9}	1.17×10^{-9}	1.9×10^{-10}	1.9×10^{-10}
		3	0.003	0.006	1.76×10^{-9}	3.52×10^{-9}	2.8×10^{-10}	5.6×10^{-10}
		6	0.003	0.007	1.76×10^{-9}	4.1×10^{-9}	2.8×10^{-10}	6.6×10^{-10}
		9	0.006	0.009	3.52×10^{-9}	5.28×10^{-9}	5.6×10^{-10}	8.4×10^{-10}
		12	0.009	0.010	5.28×10^{-9}	5.87×10^{-9}	8.4×10^{-10}	9.4×10^{-10}
		15	0.011	0.015	6.45×10^{-9}	8.8×10^{-9}	1.0×10^{-9}	1.4×10^{-9}
2	2	0	0.001	0.001	5.8×10^{-10}	5.8×10^{-10}	9.2×10^{-11}	9.2×10^{-11}
		3	0.001	0.002	5.8×10^{-10}	1.17×10^{-9}	9.2×10^{-11}	1.9×10^{-10}
		6	0.001	0.005	5.8×10^{-10}	2.93×10^{-9}	9.2×10^{-11}	4.7×10^{-10}
		9	0.002	0.005	1.17×10^{-9}	2.93×10^{-9}	1.9×10^{-10}	4.7×10^{-10}
		12	0.003	0.007	1.76×10^{-9}	4.1×10^{-9}	2.8×10^{-10}	6.6×10^{-10}
		15	0.003	0.007	1.76×10^{-9}	4.1×10^{-9}	2.8×10^{-10}	6.6×10^{-10}

For sample 1, the $CDI_{room\ temp}$ ranges room 1.17E-9 – 6.45E-9, $CDI_{sunlight}$ ranges 1.17E-9 - 8.80E-9. While the cancer risk for both exposures (room temperature and sunlight) range from 1.90E-10 – 1.0E-9 and 1.9E-10 – 1.4E-9 respectively.

For sample 2, $CDI_{room\ temp}$ and $CDI_{sunlight}$ ranged from 5.8E-10 – 1.7E-9 and 5.8E-10 - 4.1E-9. The cancer risk for room temperature and sunlight storage ranged from 9.2E-11 – 2.8E-10 and 9.2E-11 – 6.6E-10.

Exposure duration (weeks of storage) and storage temperature are the major factors determining the amount of antimony (Sb) leached from the PET bottles. These factors in addition to the concentration of leached antimony controls the cancer risk. The cancer risk increases as the storage duration, temperature and amount of leached increases. Bottling and packaging of soft drinks with PET bottles can contribute to a variety of chemical contaminants. Organic compounds such as toluene, cyclohexane, dichloromethane, pentane, benzene, phthalate, esters and ethers with tumor inducing properties may leach from plastic packaging, polystyrene cap liners or unknown sources[9].

Leaching of volatile and semi volatile organic compounds from packaging material into the soft drink has been shown to increase with length of storage time, temperature and exposure to sunlight. Because sellers of soft drinks buy in large quantities and store for a long time, at the time of purchase and consumption, the consumers are already consuming large quantities of leached antimony, additional effect is that because Nigeria is within the tropic legion, high evapo-transpiration and perspiration, the populace especially children consume large volume of soft drinks implying large quantities of leached antimony.

USEPA, (1996) states that an excess cancer risk can be negligible for values lower than 1.0E-6 whereas values above 1.0E-4 need serious attention. In general, USEPA considers excess cancer risks that are below 1 chance in 1,000,000 (1.0E-6) to be so small as to be negligible and risks above 1 in 10,000 (1.0E-4) is a risk benchmark.

The value of the present study can be considered very negligible, but the cumulative affect of long term consumption and additional effect other tumor – inducing organic contaminants can be detrimental to health.

The total cancer risk for the analyzed samples was calculated and the results were shown in [Table 3].

Table 3. The total cancer risk for the analyzed samples.

s/n	Sample	Total cancer risk$_{room\ temp}$= $\sum^{n}_{k=1} CDI_k \times SF_k$	Total cancer risk $_{sunlight}$ = $\sum^{n}_{k=1} CDI_k \times SF_k$
1	1	3.15E-9	3.33E-8
2	2	1.85E-9	3.37E-9

The total cancer risk of all the samples exposed to room temperature and sunlight correlated with each other except sample 1 in exposure to sunlight with total cancer risk of 3.33E-08 while others were in negative exponent (nine) 9.In comparison, 75% of all the samples have higher total cancer risk at exposure to sunlight than at room temperature. US EPA (1996) states that an excess cancer risk can be negligible for values lower than1.0 E-6 whereas above 1.0 E-4 are of health risk concern. Therefore, cancer risk greater than 1 in 10,000 (TCR>1) is a bench mark for gathering additional information, whereas 1/1000 or greater is a moderate increased risk and must be considered a high risk priority public health issue [10].In this study, since exposure is only through ingestion, the values were within the low risk or no risk range implying that there is low appreciable risk of cancer from exposure to antimony by drinking bottled beverages through leaching from PET bottles but when other sources such as from air, foods, water etc are summed up, the risk could be higher.

Conclusion

In this study, the antimony concentration of the carbonated soft drinks increased with storage time. The antimony concentration in samples exposed to sunlight increased faster than those stored at room temperature. The specific gravity, TSSC and acidity of the soft drinks increased with storage time, while the pH decreased with storage time.

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