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# **Effects of Direct and Indirect Sunlight on Polythene Packs, Sensory, Microbial and Chemical Properties of Sachet Water**

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#### *Authors' contributions*

*This work was carried out in collaboration among all authors. Authors WFO and AOF contributed to the execution of research work and reviewed the first draft of manuscript. Author OMA established the experimental design and coordinated water analysis. Author AA reviewed the first draft of the article and contributed to the publication process. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The use of Polyethylene or Polyethylene terephthalate (PE/PET) packaging bags for water in Nigeria has great marketing appeal to consumers. However, poor storage and display techniques could subject these products to microbial, physical and chemical deterioration. Sachet water samples commonly taken by staff and students of the Federal College of Agriculture, Ibadan were subjected to different storage conditions for three and five days. Physicochemical parameters of each water sample were determined through equilibrated devices and titrimetric or colorimetric assays, while aerobic bacteria were isolated through pour-plate method in Nutrient Agar. Exposure of sachet water to sunlight had significant effects on their physical, chemical and bacteriological properties. Highest chloride, calcium, alkalinity, hardness, sulphate and total dissolved solids (13.04 mg/L, 123.53 mg/L, 9.08 mg/L, 33.00 mg/L, 27.59 mg/L and 78.33 mg/L, respectively) were recorded in water samples exposed to indirect sunlight for 5 days. Samples stored at room temperature had the best physicochemical properties. Exposure to sunlight reduced the colony forming units of aerobic bacteria in all the water samples. Lowest bacterial count (0.33  $\times$  10<sup>3</sup> CFU/mL) was observed in water sample exposed to sunlight for 5 days, while the highest count

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(55.84 CFU/mL) was recorded in samples stored at room temperature. Sensory scores of water samples ranged between 8.33 and 10.00; however, these sensory properties were not significantly affected by their exposure to sunlight for up to 5 days. Sunlight exposure negatively affected the inner surface feel of water packs, sachets appeared slimy after direct and indirect exposure. The physicochemical and microbial changes observed in sunlight-exposed sachet water samples did not influence their organoleptic acceptability. Consequently, in addition to taste and biochemical analysis, the feel of water sachets could be an indication of structural disintegration and water contamination. In order to maintain the integrity of sachet water, products should be hygienically prepared and prevented from sunlight exposure during transportation and storage.

*Keywords: Water; physicochemical; sensory; sunlight; sachet integrity.*

### **1. INTRODUCTION**

Adequate consumption of clean, safe and potable water is a basic requirement for humans to maintain good health and balance the body fluids. Metabolic fluids are generally essential for digestion, streaming of body nutrients, circulation and to stabilize the body temperature [1]. Water is generally packaged in a number of presentations; these include sachet, PE/PET premade bag or bottles, glass packs and plastic cups and bottles. In Nigeria, production of water in sachet is a cheaper and supposedly safer way of making portable (drinking) water readily available to consumers at affordable rates [2]. With the aid of regulatory agencies, like the<br>National Agency for Food and Drug National Agency for Food and Drug Administration and Control, most of the sachet water producing factories are guided towards the production of safe drinking water. However, asides the evasive nature of some factory owners to boycott standard procedures, such bagged water products are subjected to varying degrees of post-packaging environmental conditions, during transportation, storage and display. Within Ibadan metropolis, packed bags of sachet water are often transported and subsequently displayed under direct and indirect sunlight.

Ultraviolet rays from sunlight radiate energy which significantly speeds up photochemical degradation reactions and affects the stability of photosensitive packaging materials like the PE/PET [3]. By extension, polymers of these packaging materials tend to undergo oxidative reactions when exposed to sunlight, leaching chemical products into water. This in turn results in microplastic contamination, as well as physical and chemical alterations like discolouration and weakening of the packaging polymer [4]. The bacteriological quality of drinking water is one of the most important environmental parameters for the prevention and control of waterborne diseases in both developed and developing countries like Nigeria. The contamination of drinking water by pathogens and opportunistic organisms could occur within the distribution system, unhygienic handling (through contact with hands and utensils), as well as through storage and packaging materials [5].

Recent outbreaks of waterborne diseases in Nigeria have been linked to the consumption of polluted water. In August 2017, an occurrence of waterborne disease outbreak started in Muna Internally Displaced Persons camp, Borno State, Nigeria, with over 5000 cases, cutting across six local government areas [6]. A similar contaminated water-related outbreak of disease was reported in Adamawa State (Nigeria) recently, wherein a total number of cases documented at 8th November, 2019 stood at 818 with 4 deaths [7]. These waterborne diseases could readily lead to death, especially in circumstances where correct and adequate treatment is neither readily available nor affordable [8]. The type and concentration of contaminants in water could affect the sensory properties and acceptability of such water source. Groundwater is usually contaminated with magnesium, calcium, chloride, arsenate, nitrate, sulphate and iron. The combinations of these contaminants therefore influence the taste and aftertaste of water [9]. There have been studies on the impact of sunlight on sachet water properties [10,11,12]; however, more information is required on the physical response of these polythene packs to radiation from the sun, as it relates to organoleptic, physicochemical and bacteriological properties of water. This research is therefore designed to investigate the effects of direct and indirect display of water under sunlight on the sensory, microbial, physical and chemical properties of sachet water.

## **2. MATERIALS AND METHODS**

#### **2.1 Sample Collection and Storage**

Three (3) most commonly consumed sachet water (PE/PET bagged drinking water) by students of the Federal College of Agriculture, Ibadan were collected from their respective production industries. They were labeled AQBE, SFHE and ORSE, denoting their brand names and location of their stations. Samples were collected from portable-water packaging plants (with groundwater source) at Apata, Oluyole and Eleyele areas of Ibadan, Oyo State, Nigeria. These samples were collected at the day of production and transported to the laboratory for immediate analysis (control) or storage under different treatment conditions for three and five days. Exposure to sunlight was carried out from  $1<sup>st</sup>$  to  $5<sup>th</sup>$  January, 2021 as highlighted below:

- storage at room temperature (25±3.57ºC)
- exposure to indirect sunlight between 12:00 and 4:00 pm daily (covered with a blanket, under sunlight, as sometimes done by producers during distribution and storage)
- exposure to direct sunlight between 12:00 and 4:00 pm daily.

#### **2.2 Physicochemical Analysis of Water**

Each treated water sample, subjected to different storage conditions, was retrieved for analysis. Properly washed, pre-sterilized pasture's pipettes were used to collect water samples for both physical and chemical analyses. The calcium  $(Ca^{2+})$ , magnesium  $(Mg^{2+})$ , chloride (Cl<sup>−</sup>), sulphates  $(SO<sub>4</sub><sup>2−</sup>)$ iron  $(Fe^{2+})$ , electrical conductivity, alkalinity, hardness, dissolved solid levels, as well as the pH of the water samples were determined based on standard procedures.

#### **2.2.1 Total dissolved solids (TDS), hardness and pH**

Glass fibers were soaked in sterile distilled water and thereafter dried at 103ºC. These fibers were weighed and the weights were recorded as their initial weights. Glass fibers were carefully placed in a filtering flask. Sachet water samples were shaken to evenly distribute dissolved materials within each sample and then poured into the prepared filtering flask. For each sachet water sample, 200 mL of water was filtered through the pump. The filter within the flask was then retrieved, dried to constant weight at 103ºC, cooled at room temperature and reweighed to determine the final weight. The difference in the weight of glass fiber represented the increase in weight as a result of solids within the given volume of water samples [11]. Total hardness (TH) was determined through the ethylene diamine tetra-acetic acid (EDTA) titrimetric method, using Eriochrome black-T as an indicator [13]. The measure of hydrogen ion concentration in the water samples subjected to different storage conditions was determined using an equilibrated electromagnetic logger with pH probe, according to manufacturer's instructions.

#### **2.2.2 Determination of other chemical parameters and electrical conductivity**

Sulphates were determined through turbidimetric analysis with barium chloride  $(BaCl<sub>2</sub>)$  as precipitant. Water sample (50 mL) was measured into a 500 mL beaker and diluted to 150mL with sterile distilled water. Thereafter, concentrated HCl (1 mL) and four drops of indicator (methyl orange) were added to the sample. Precipitant solution (10 mL of 10%  $BaCl<sub>2</sub>$ ) was then added and boiled for 5 minutes. The resulting solution was left to stand overnight and thereafter filtered using Whatman filter paper. The filter paper was rinsed with distilled water (to release attached chloride ions) and dried at 80°C in an oven. Filter paper, in each case, was dried with silica crucible, ignited at 800 $^{\circ}$ C in a mu $\Box$ e furnace for 30 minutes, cooled in a desiccator, and weighed afterwards. This process was repeated till a constant weight was recorded on subsequent analysis. Sulphate content of each sachet water sample was then determined [14].

Total iron content of water was determined through colorimetric assay method using the spectrophotometer, while the calcium, magnesium and chloride content of these samples were determined by titrimetric methods (with digital titrator) as described in the American Public Health Association (APHA) standard methods for the examination of water [15,16,14]. The conductivity of sachet water samples was measured using a conductivity device (conductivity meter). The sensitive probe of this meter was calibrated using standard solutions with predetermined conductivity. Calibrated probe was thereafter submerged in water sample, allowed to stabilize and the conductivity displayed was recorded. All the reagents used for the analysis were of analytical grade, while

the instruments were in the limits of precise accuracy [16].

#### **2.3 Bacteriological Analysis**

Each water sample was serially diluted (10 $^6$ ) and introduced to sterile Petri Dish. Nutrient Agar, sterilized at 121ºC for 15 minutes at 15 psi, was cooled to 45ºC and then poured over the sample in each plate (pour plate isolation). Inoculated plates were incubated at room temperature (25±2ºC) for 24-48 hours. Growing colonies were counted using a colony counter and the aerobic bacterial count was estimated from the dilution factor of the original sample [17].

#### **2.4 Organoleptic Analysis**

Water samples were assessed for their sensory properties at the Home Science Kitchen of the Federal College of Agriculture, Ibadan by a panel of trained tasters. Each of the participants took 30 mL of water sample in a graduated glass cup, passing it over the tongue surface and thereafter recording the results for each sensory parameter. A ten point hedonic scale evaluation was adopted for the scoring, wherein the tasters were required to score their satisfaction upon tasting on the scale from 1 (Dislike extremely) to 10 (Like extremely) [18]. The feel of the internal surface of each empty sachet was also reported by the taste panel as very slimy, slimy or not slimy. This was done to establish the possible effect of sunlight and storage duration on the physical integrity of these polyethylene terephthalate packaging materials.

Data replicates were subjected to analysis of variance and the means of each treatment were separated using the Duncan's Multiple Range Test (DMRT) at 95% level of confidence.

#### **3. RESULTS AND DISCUSSION**

### **3.1 Effect of Sunlight on the Physicochemical Properties of Sachet Water**

Direct and indirect exposure of sachet water to sunlight significantly influenced the measure of physicochemical parameters in selected water samples. Total hardness ranged from 0.00 (in AQBE and ORSE water samples) to 33.00 mg/L (in SFHE water sample exposed to sunlight for 5 days), hardness increased in all sachet water samples with longer storage period (Table 1). Hardness in water is an indication of the

availability of metal ions. Ahmed *et al.* [19], in their study on drinking water quality mapping, using water quality index and geospatial analysis, reported an average hardness concentration of 291 mg/L. Highest total hardness, in this present study, was observed in SFHE water sample exposed to indirect sunlight for 5 days. However, this was still within the range of acceptable hardness level in drinking water (300 mg/mL), as established by the World Health Organisation [20,21].

The levels of total dissolved solids (TDS) in the water samples were relatively low, ranging from 30.67 to 78.33 mg/L. The World Health Organisation reported total dissolved solids levels between 0 and 900 mg/mL as acceptable, while drinking water with best qualities have TDS levels lower than 500 mg/mL [22]. Calcium content of sachet water samples varied widely between 20.00 and 123.53 mg/L. Highest calcium content was recorded in SFHE water sample exposed to sunlight for 5 days. This sample (SFHE) also had the highest alkalinity and sulphate content (9.08 and 27.59 mg/L, respectively), recorded in water exposed to indirect sunlight for 5 days. The recorded values of calcium, sulphate and alkalinity, in these water samples, were within the acceptable limits of < 200 mg/L, 250 mg/L and 200 mg/L, respectively [16,20,21]. Water stored at room temperature had relatively stable levels of chemical and physical properties. There was no significant difference in the levels of chloride, calcium, alkalinity, sulphate, dissolved solids, magnesium, iron and conductivity rates for the selected water samples stored for three and five days at room temperature.

Changes in the physicochemical properties of sachet water exposed to sunlight could have been as a result of chemical leach induced by ultraviolet radiation. This could in turn be an indication of PEP package disintegration, releasing cytotoxic, mutagenic compounds into sachet water. Anayo *et al.* [12] also studied the impact of ultraviolet radiation on polyethylene packaged water and reported the release of various chemicals, such as polycyclic aromatic hydrocarbons, trichloromethane, benzene, limonene, xylene, toluene and 2-hexanone into sachet water exposed to direct sunlight. This was however contrary to the report of Ikpeazu and Oluwayiose [10], who studied the effect of ultraviolet radiation on sachet water and reported quality improvement of bagged water through sunlight exposure.

## **3.2 Bacteriological Analysis of Sachet Water**

As presented in Table 2, all the selected water samples (control) exceeded the WHO permissible limits for total heterotrophic bacterial count in drinking water (<500 CFU/mL) [21,17]. Exposure to sunlight reduced the colony forming units of aerobic bacteria in all the water samples. The lowest bacterial count (0.33  $\times$  10<sup>3</sup> CFU/mL) was observed in AQBE water sample exposed to indirect sunlight for 5 days (Table 2). Aerobic bacterial units in SFHE and ORSE water samples exposed to direct sunlight reduced by 16.66  $\times$  10<sup>3</sup> CFU/mL and 51.51  $\times$  10<sup>3</sup> CFU/mL, respectively. Pullerits *et al.* [23] also studied the impact of ultraviolet (UV) irradiation on bacterial communities in drinking water. Samples, in their study, were irradiated with ultraviolet doses of 250, 400, and 600 J/ $m^2$ , and the effect on bacterial communities was subsequently investigated using 16s rRNA gene amplicon sequencing and heterotrophic plate counts (HPCs). They reported the reduction in bacterial families such as *Chitinophagaceae, Comamonadaceae,* and *Flavobacteriaceae* after water irradiation. The mechanism of disinfection or reduction in bacterial population resulting from exposure to sunlight could be attributed to nucleic acids damage by irradiation. Nucleotides have been reported to absorb UV light with wavelengths between 200 and 300 nm, with peak absorption between 260 and 265nm. This could trigger the formation of mutagenic DNA lesions, blocking DNA replication, resulting in the inactivation of microbial cells [24].

## **3.3 Sensory Analysis of Exposed Sachet Water**

Inner surface change was observed on water packs exposed to both direct and indirect sunlight. The most noticeable change was observed in SFHE and ORSE water samples exposed to indirect sunlight for 5 days (Table 2). Polythene packs appeared very slimy to touch after sunlight exposure. Only ORSE sachet felt slimy at room temperature storage, AQBE water pack also appeared slimy after indirect exposure to sunlight for 5 days. Changes in the inner surface feel of polythene packs may be as a result of photochemical reactions induced by sunlight. The polyethylene material used for sachet water packs could undergo photolytic, photo-oxidative and thermo-oxidative reactions when exposed to sunlight over a period of time.

This could, in turn, result in the fragmentation of polyethylene materials, altering its structural integrity and leaching its components into water [12]. The observed slimy inner surface feel of polythene packs could also be as a result of microbial contaminants within the packaged water, forming a slimy, biofilm attachment [25] on the pack surface. Exposure of water packs to sunlight could increase the rate of PEP degradation, generating microplastics. These microplastics could in turn encourage biofilm complex formation [26,25] around the inner surface of exposed polythene packs. The formation of bacteria-microplastic complexes, especially biofilms, enhances horizontal gene transfer among microorganisms [26]. The transfer of genes, such as the antimicrobial resistance, diverse metabolic pathways and pathogenicity genes, could result in yet another global health challenge. Although, sunlight exposure reduced the CFU/mL of bacterial count in the sachet water samples; however, this appeared insufficient to prevent the possible development of biofilm complex resulting from sunlight-induced PEP degradation. Indirect sunlight appeared to have the most significant effect on the integrity of water packs, as well as the physicochemical properties of sachet water. This could be as a result of the combined effects of radiation from sunlight and the resultant heat trapped within the blanket cover on these water samples [27].

Exposure of sachet water to both direct and indirect sunlight appeared not to significantly affect the sensory (taste, odour and colour) parameters of these water samples (Table 3). The measure of sensory properties of sachet water exposed to direct and indirect sunlight for 3 and 5 days ranged from 8.33 to 10.00; these were not significantly different from values recorded for the control in each case. However, the colour of AQBE sachet water sample exposed to indirect sunlight for 3 and 5 (8.67 and 8.00 respectively) was less desirable. Changes in the physicochemical properties of water could affect the taste and acceptability of such water source. However, within permissible range of physical and chemical changes of water, its palatability might be unaffected [22]. Sharma and Bhattacharya [9] reported physicochemical parameters, storage conditions, as well as the by-products of industries, laboratories and agriculture as factors that could significantly affect the taste and acceptability of drinking water. These parameters include heavy metals like mercury, copper, chromium, lead, dyes and

<b>Parameter</b>	Room temperature				Indirect sunlight			<b>Direct sunlight</b>		
	S.D	<b>AQBE</b>	<b>SFHE</b>	<b>ORSE</b>	<b>AQBE</b>	<b>SFHE</b>	<b>ORSE</b>	<b>AQBE</b>	<b>SFHE</b>	<b>ORSE</b>
Chloride (mg/L)	3	9.55	9.55	5.61	12.12	12.13a	7.13	9.55	9.55	5.61
	5	9.55	9.55	5.61	13.04	10.07b	5.93	9.71	9.67	5.80
	С	9.45	9.45	5.56	9.45	9.45b	5.56	9.45	9.45	5.56
Significance		ns	ns	ns	ns	*	ns	ns	ns	ns
Calcium (mg/L)	3	50.50	111.10	20.20	51.25	115.57	20.79a	50.50ab	111.10	20.20ab
	5	50.50	111.10	20.20	51.50	123.53	20.10b	50.77a	111.77	20.43a
	C	50.00	110.00	20.00	50.00	110.00	20.00b	50.00b	110.00	20.00b
Significance		ns	ns	ns	ns	ns	$\star$	$\star$	ns	ns
Alkalinity (mg/L)	3	5.22	8.08	4.71	3.95c	6.81c	3.44b	5.22	8.08a	4.71
	5	5.22	8.08	4.71	7.55a	9.08a	5.75a	5.33	8.09a	4.94
	$\mathsf{C}$	5.17	8.00	4.67	5.50b	8.00 <sub>b</sub>	4.67a	5.17	8.00b	4.67
Significance		ns	ns	ns		$\star$	*	ns	$\star$	ns
Hardness (mg/L)	3	0.00 <sub>b</sub>	12.67b	0.00 <sub>b</sub>	1.21 <sub>b</sub>	17.33b	1.05b	0.00 <sub>b</sub>	12.67b	0.00 <sub>b</sub>
	5	2.05a	17.67a	1.03a	6.19a	33.00a	5.08a	2.06a	17.33a	1.04a
	$\mathsf{C}$	0.00 <sub>b</sub>	12.67b	0.00 <sub>b</sub>	0.00c	12.67c	0.00c	0.00 <sub>b</sub>	12.67b	0.00 <sub>b</sub>
Significance		$\star$	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$
Sulphate (mg/L)	3	12.28	22.62	1.96	11.27	21.61b	0.95 <sub>b</sub>	12.28	22.62	1.96
	5	12.28	22.62	1.96	12.74	27.59a	2.04a	12.49	26.00	2.01
	С	12.16	22.40	1.95	12.16	22.40b	1.95a	12.16	22.40	1.95
Significance		ns	ns	ns	ns	$\star$	$\star$	ns	ns	ns
<b>TDS</b>	3	42.33	62.67	30.67	45.33b	66.00b	32.33b	43.33	63.00	31.00
(mg/L)	5	42.33	62.67	31.33	60.33a	78.33a	54.00a	42.33	63.67	31.00
	C	41.33	60.33	30.67	41.33b	60.33b	30.67b	41.33	60.33	30.67
Significance		ns	ns	ns	$\star$	$\star$	$\star$	ns	ns	ns
pH	3	6.08	6.76	6.06	5.83b	6.78b	5.06c	6.08	6.83	6.05
	5	6.08	6.83	6.05	6.85a	6.98a	6.12a	6.07	6.83	6.07
	С	6.10	6.83	6.05	6.10b	6.83 <sub>b</sub>	6.05 <sub>b</sub>	6.10	6.83	6.05
Significance		ns	ns	ns	$\star$	$\star$	$\star$	ns	ns	ns

**Table 1. Physicochemical properties of selected sachet water samples**

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*Mean values with similar letter(s) down the column are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT); S.D: Storage day(s); TDS: Total dissolved solids; C: Control, ns: not significant; \*: significant*

#### **Table 2. Effect of sunlight on the microbial properties and inner surface feel of water sachets**



*Mean values with similar letter(s) down the column are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT); S.D: storage day(s); AMC: aerobic microbial count; C: control; ISF: inner surface feel of water sachets; -: not slimy; +: slimy; ++: very slimy; ns: not significant; \*: significant*



## **Table 3. Effect of sunlight on sensory properties of sachet water**

*Mean values with similar letter(s) down the column are not significantly different at 5% level of significance by Duncan's Multiple Range Test (DMRT); C: Control; ns: Not significant; \*: Significant*

compounds like insecticides, fertilizers, cytotoxic synthetic materials, as well as microorganisms and their extracellular metabolites [9].

## **4. CONCLUSION**

Higher levels of chemical compounds, lower aerobic bacterial count and slimy inner surface feel of water packs were recorded in sachet water samples exposed to direct and indirect sunlight, compared to samples stored at room temperature. However, physicochemical, biological and possible structural changes in polyethylene packed water samples did not influence their sensory properties. This could make it difficult for unsuspecting consumers to identify sachet water subjected to poor display or storage conditions by their taste. Physical properties of these packs could also aid towards on-the-spot assessment of their structural integrity. Water should be properly disinfected through the production systems, in order to eliminate contaminants or reduce microbial count to acceptable levels before bagging. It is also imperative to enforce water production standards by regulatory agencies. In situations where products cannot be refrigerated, sachet water vendors should be encouraged to store them at room temperature (away from sunlight). Deliveries should also be made to wholesalers early in the morning or later in the evening to limit or avoid exposure to radiation.

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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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