UNIOFFA JOURNAL OF INTERDISCIPLINARY STUDIES (U-OJIS)
A Multidisciplinary Journal of Science, Management and Social Sciences
Website: www.unioffajis.com
ISSN:3141-5571, E-ISSN:3141-558X

EFFECT OF STORAGE CONDITIONS ON THE MICROBIOLOGICAL QUALITY OF SACHET WATER PACKAGED IN POLYETHYLENE IN OFFA AND OYUN LOCAL GOVERNMENT AREAS, KWARA STATE, NIGERIA

Akinboboye Olusola Arinola
Department of Microbiology, Southwestern University Nigeria, Okun-Owa, Ijebu-Ode, Ogun State, Nigeria.

Pedro Uwadum Adagbor
[1]Department of Microbiology, Southwestern University Nigeria, Okun-Owa, Ijebu-Ode, Ogun State, Nigeria.

Corresponding author: Puwadum@gmail.com

VOLUME 2:1

Abstract

Sachet water packaged in polyethylene is a major source of drinking water in Nigeria, particularly in communities where access to potable water remains limited. Although packaged water is expected to meet microbiological safety requirements at production, its quality may change during storage. This study examined the effect of storage conditions and duration on the microbiological quality of sachet water in Offa and Oyun Local Government Areas of Kwara State, Nigeria. A controlled laboratory experimental design was adopted. Four sachet-water brands were subjected to four storage conditions: direct sunlight, shaded ambient conditions, controlled room temperature (25 ± 2°C), and refrigeration (4 ± 2°C). Samples were analysed at baseline and after 3, 7, 14 and 28 days, with three replicates for each experimental combination, giving 240 sachet-water units. Microbiological quality was assessed using total heterotrophic bacterial count, total coliform count and detection of Escherichia coli*, while pH was monitored as a supporting parameter. Data were analysed using descriptive statistics and factorial analysis of variance at* $p < .05$*. The findings showed that storage condition significantly influenced microbiological quality. Mean heterotrophic bacterial counts were highest under direct sunlight (41.33 CFU/mL) and lowest under refrigeration (11.46 CFU/mL), while mean total coliform counts were highest under direct sunlight (11.79 CFU/100 mL) and lowest under refrigeration (3.89 CFU/100 mL). Counts increased with storage duration, with heterotrophic bacterial counts rising from 8.02 CFU/mL at baseline to 47.59 CFU/mL by Day 28. A significant interaction between storage condition and duration indicated that microbial changes over time varied according to the storage environment.* Escherichia coli *was detected in 7.9% of samples, with occurrence increasing at later storage periods, while pH remained relatively stable. The study concludes that storage environment and duration are important determinants of sachet-water microbiological quality. Protection from direct sunlight and prolonged storage is therefore important for maintaining microbiological quality.*

Keywords: sachet water, polyethylene, storage conditions, microbiological quality, coliforms, *Escherichia coli*, heterotrophic bacteria, Offa, Oyun, Kwara State.

INTRODUCTION

Access to safe drinking water remains one of the most important determinants of public health because contaminated water provides a pathway through which pathogenic microorganisms can reach human populations. The quality of drinking water is therefore not only a question of availability but also of microbiological safety throughout the period between production and consumption. Among the microorganisms used in assessing the safety of drinking water, *Escherichia coli* remains particularly important because its detection provides evidence of faecal contamination and a possible risk of the presence of enteric pathogens. The World Health Organization (WHO, 2022) recommends that *E. coli* should not be detectable in any 100 mL sample of water

ISSN:3141-5571
E-ISSN:3141-558X

intended for human consumption. This requirement reflects the principle that water may be considered unsuitable for drinking when its microbiological integrity has been compromised.

In Nigeria, the challenge of providing safe and reliable drinking water has contributed to the rapid expansion of packaged drinking water, particularly sachet water. Sachet water has become a familiar feature of everyday life in urban, semi-urban and rural communities because it is relatively inexpensive, portable and readily available. For many consumers, it represents a convenient alternative where public water supply is irregular or unavailable. Its widespread consumption, however, makes the microbiological safety of sachet water an important public-health concern. The safety of the product cannot be considered solely in relation to the quality of water at the point of production. Rather, it is a process that extends through packaging, transportation, retail storage and eventual consumption. Water that meets microbiological requirements when packaged may encounter different environmental conditions before it is consumed.

This issue is particularly important because sachet water is packaged in polyethylene and is frequently transported and stored under conditions that are not necessarily controlled. In practice, sachets may be displayed in open markets, placed beside roads, kept in shops without adequate ventilation, stacked on floors or exposed to direct sunlight for considerable periods. Such conditions may subject the product to variations in temperature and handling that are different from those under which its microbiological quality was originally assessed. The possibility of microbial changes during this period raises an important question about the extent to which the conditions of storage influence the quality of packaged drinking water.

The Nigerian regulatory framework recognises the importance of maintaining hygienic conditions in the production of packaged water. The National Agency for Food and Drug Administration and Control (NAFDAC) provides requirements relating to packaged-water facilities, production processes, hygiene and quality control. These requirements are intended to ensure that water is produced and packaged under conditions capable of protecting consumers from microbiological and other forms of contamination (NAFDAC, 2018). However, the existence of production standards does not necessarily mean that the microbiological quality of sachet water remains unchanged throughout its distribution and storage period. The distinction is important because regulatory compliance at production does not eliminate the environmental conditions to which the product may subsequently be exposed.

Empirical evidence from Nigeria increasingly points to the importance of this distinction. Enaigbe and Imade (2021), in a study of seven NAFDAC-certified sachet-water brands produced in Benin City, examined the microbiological and physicochemical characteristics of the products over an extended storage period. Their findings showed that while some parameters remained within recommended limits, two brands recorded heterotrophic plate counts above the WHO and NAFDAC recommended limit by the eighth week of storage. The study is particularly relevant because it demonstrated that microbiological quality could change during storage even where the products had been obtained directly from certified manufacturers. It consequently raised an important question about the adequacy of assuming that a uniform shelf life applies equally to all packaged-water brands under storage conditions (Enaigbe & Imade, 2021).

Studies conducted in Kwara State provide further evidence that the microbiological quality of packaged water warrants continued investigation. Jimoh, Ajao, Aborisade, Abdulsalam, and Kolawole (2025) examined packaged water sold in Ilorin and found that although the measured physicochemical parameters and heterotrophic bacterial counts of the examined samples were generally within reported permissible limits, total coliform counts exceeded the acceptable range. The researchers also isolated organisms including *Pseudomonas aeruginosa*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella* spp. and *Bacillus subtilis*. Their findings demonstrate that packaged-water safety remains a relevant microbiological issue within Kwara State and that compliance in some physicochemical parameters does not necessarily translate into complete microbiological safety (Jimoh et al., 2025).

Similarly, Balogun et al. (2024) investigated polyethylene-packaged groundwater in the

ISSN:3141-5571
E-ISSN:3141-558X

Ilorin metropolis and reported variations in the microbiological and physicochemical characteristics of the sampled products. Their study focused principally on determining the quality of polyethylene-packaged groundwater and its conformity with established standards. This contributes useful evidence on the quality of packaged water within the broader Kwara State environment, but, like most point-of-sale quality assessments, it provides limited information about what happens to the microbiological characteristics of the water after it has remained under different storage conditions for varying periods.

The situation is also relevant to Offa and Oyun Local Government Areas. Earlier research on drinking-water sources in selected communities within the two Local Government Areas identified total and faecal coliforms in some of the examined water sources, suggesting that microbiological contamination remains an important consideration in the local drinking-water environment (Olawale, Awe, & Salako, 2019). More recent work in Offa has similarly reported microbiological and physicochemical concerns in drinking-water sources, reinforcing the need for continued local investigation of water safety (World Journal of Advanced Research and Reviews, 2024). These studies are important because they establish that the question of microbiological water quality is not remote from the communities under investigation. Nevertheless, the evidence generated from these studies largely concerns the quality of water sources at the point of examination rather than the changes that may occur in commercially packaged sachet water as it passes through different storage environments.

This distinction reveals an important methodological and empirical gap in the existing literature. Previous studies have made valuable contributions by determining the microbiological quality of sachet water, identifying bacterial contaminants, assessing compliance with regulatory standards and, in the case of Enaigbe and Imade (2021), examining changes in selected sachet-water brands over an extended shelf-life period. However, there remains limited experimental evidence on the comparative effect of specific storage conditions and storage duration on sachet water sold within Offa and Oyun Local Government Areas. Existing studies do not adequately explain whether exposure to direct sunlight, storage under shaded ambient conditions, controlled room temperature or refrigeration produces different microbiological outcomes over time. They also provide limited evidence on the interaction between storage condition and duration. Consequently, it remains unclear whether changes observed in packaged water are attributable primarily to the passage of time, the environmental conditions under which the product is stored, or the combined effect of both factors.

The gap is significant because the conditions under which consumers purchase sachet water are often substantially different from those prevailing in a controlled production environment. A sachet of water may leave a production facility in acceptable microbiological condition but subsequently spend several days or weeks in a retail environment exposed to elevated temperature, sunlight or repeated handling. Thus, assessing microbiological quality only at the point of production or purchase may provide an incomplete picture of the conditions under which consumers ultimately consume the product. The problem is not simply whether sachet water contains microorganisms at a particular point in time, but whether its microbiological characteristics change as a consequence of how and for how long it is stored.

The present study responds to this gap by moving beyond a conventional point-in-time assessment of sachet-water quality to a controlled examination of microbiological changes under defined storage conditions. It focuses specifically on sachet water packaged in polyethylene and commercially available in Offa and Oyun Local Government Areas of Kwara State. By examining microbial indicators at different storage intervals under contrasting environmental conditions, the study seeks to establish whether storage condition and duration significantly influence microbiological quality. Particular attention is given to total heterotrophic bacterial count, total coliforms and *E. coli*, which together provide information on general bacterial load and potential faecal contamination.

The significance of this approach lies in its ability to connect laboratory evidence with the actual circumstances under which sachet water is handled and consumed. Rather than assuming that the microbiological status of

ISSN:3141-5571
E-ISSN:3141-558X

sachet water remains constant after production, the study treats storage as an important stage in the water-safety chain. The evidence generated will therefore provide a basis for understanding whether particular storage practices are associated with greater microbiological deterioration and whether prolonged storage alters the safety status of the product. Such evidence is relevant not only to consumers but also to sachet-water producers, retailers, public-health practitioners and regulatory agencies concerned with maintaining the safety of packaged drinking water in Nigeria. Accordingly, this study investigates the effect of storage conditions on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State, Nigeria. The central concern is to establish empirically whether the microbiological quality of sachet water changes with storage duration and whether the magnitude and direction of such changes differ according to the environmental conditions under which the water is stored.

Research objective

1: To determine the effect of different storage conditions on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State.

2: To determine the effect of storage duration on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State.

Research Questions

What effect do different storage conditions have on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State?

What effect does storage duration have on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State?

Research Hypotheses

The hypotheses should correspond exactly to the two research questions:

H_{01}: Storage conditions have no significant effect on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State.

H_{02}: Storage duration has no significant effect on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State.

LITERATURE REVIEW

Conceptual Review

1 Sachet Water

Sachet water is drinking water that is processed and packaged in sealed polyethylene sachets for commercial distribution and human consumption. The concept of sachet water, however, extends beyond the physical packaging of water. It represents a form of commercially produced drinking water that passes through a number of stages from water sourcing and treatment to packaging, transportation, storage, retailing and final consumption. Consequently, the quality of sachet water at the point of consumption may reflect conditions occurring at different stages of the production and distribution chain. In Nigeria, sachet water has become an important component of the drinking-water system because of its affordability, portability and accessibility. Its widespread consumption has also generated concern about the maintenance of microbiological quality after production. The fact that water has undergone treatment and has been packaged under regulated conditions does not necessarily mean that its microbiological quality will remain unchanged throughout its period of storage and distribution. The conditions under which the packaged water is transported, displayed and stored can influence the quality eventually available to consumers.

Different dimensions can therefore be identified in the conceptualisation of sachet water. These include the source of the water, treatment process, packaging material, hygienic conditions during production, storage conditions, duration of storage, distribution practices and microbiological quality. The present study is particularly concerned with the storage stage because it represents the period during which packaged water remains outside the immediate control of the production process before consumption.

ISSN:3141-5571
E-ISSN:3141-558X

Sachet water can consequently be conceptualised in this study as a treated drinking-water product packaged in polyethylene and subjected to environmental and temporal conditions between production and consumption. This conceptualisation is important because the study does not examine the general acceptability of sachet water, but specifically investigates whether conditions under which the product is stored can alter its microbiological quality.

2 Polyethylene Packaging and Sachet-Water Safety

Polyethylene is widely used in the packaging of sachet water because it is relatively lightweight, flexible, inexpensive and suitable for forming sealed packages. Its use provides a physical barrier between the water and the external environment. However, the presence of a polyethylene package does not by itself guarantee microbiological safety. The relationship between packaging and water quality has several dimensions. The first is the integrity of the package. A properly sealed sachet can reduce the possibility of external contamination, while defective or damaged packaging can provide an opportunity for microorganisms to enter the product. The second dimension is handling and hygiene. Contamination may occur when packaged water comes into contact with contaminated hands, surfaces, transportation containers or retail environments. The third dimension concerns the environmental conditions surrounding the package. Temperature, sunlight and duration of storage may influence the survival or multiplication of microorganisms already present in the water.

Packaging should therefore be understood as one component of a broader production-to-consumption system. The microbiological quality of sachet water is not determined exclusively by the material from which the sachet is produced. It is also influenced by the quality of the source water, treatment effectiveness, hygienic practices, package integrity, environmental exposure and post-production handling. This position is consistent with empirical evidence from Nigeria. Enaigbe and Imade (2021), in their assessment of NAFDAC-certified sachet-water brands, demonstrated that microbiological quality could change during storage even where the products had undergone regulated production processes. Similarly, studies of packaged water in Ilorin have reported variations in bacteriological quality among commercially available water products, indicating that packaged status does not necessarily imply uniform microbiological quality (Balogun et al., 2024; Jimoh et al., 2025).

For the present study, polyethylene packaging is therefore conceptualised as the physical containment system within which treated drinking water is stored, rather than as the independent determinant of microbiological quality. The study is particularly interested in what happens to the packaged water when it is subjected to different storage environments over different periods.

3 Microbiological Quality of Drinking Water

Microbiological quality refers to the condition of water in relation to the presence, absence, concentration and behaviour of microorganisms that may indicate contamination or pose a potential health concern. It is one of the principal dimensions through which drinking-water safety is assessed. Unlike physical characteristics such as colour and turbidity or chemical characteristics such as pH and dissolved substances, microbiological quality focuses specifically on microorganisms and microbiological indicators associated with water safety. The concept has several dimensions. These include microbial presence, microbial concentration, indicator organisms, pathogenic organisms and changes in microbial population over time. In drinking-water assessment, indicator organisms are particularly important because testing for every possible pathogen is neither practical nor necessary for routine monitoring. Total coliforms and Escherichia coli are therefore commonly used as indicators of microbiological contamination, while heterotrophic bacterial counts can provide information about the general bacterial population present in a water sample.

The concept of microbiological quality is consequently broader than simply determining whether bacteria are present. The concentration and type of microorganisms are also important. A water sample may contain detectable bacteria without necessarily having the same level of microbiological concern as another sample containing a substantially higher microbial population or organisms associated with faecal contamination. The World Health Organization (WHO) places

ISSN:3141-5571
E-ISSN:3141-558X

particular importance on E. coli as an indicator of faecal contamination in drinking water. Its detection in drinking water is therefore an important microbiological quality concern. Total coliforms can provide broader information about sanitary conditions, while heterotrophic bacterial counts can be used to examine the general bacterial population and changes occurring during storage.

In this study, microbiological quality is operationalised through total heterotrophic bacterial count, total coliform count and the detection or occurrence of E. coli. These indicators provide three related but distinct dimensions of microbiological assessment. Total heterotrophic bacterial count provides an indication of the general bacterial population; total coliform count provides information relevant to sanitary quality; and *E. coli* provides a more specific indication of faecal contamination. The adoption of these indicators is important because the study is concerned not merely with whether sachet water remains visually acceptable during storage, but whether its microbiological characteristics change under different storage conditions and over different periods.

4 Storage Conditions of Sachet Water

Storage condition refers to the environmental circumstances under which a product is kept between production and consumption. In the context of sachet water, storage conditions include factors such as temperature, exposure to sunlight, shade, ventilation, surrounding environment and refrigeration. These conditions can influence the physical and microbiological characteristics of packaged water.

Storage condition is particularly important because microorganisms respond differently to environmental conditions. Temperature, for example, affects microbial metabolic activity and reproduction. Light exposure may also contribute indirectly to changes in the water environment through heating of the package and associated changes in temperature. Refrigeration, on the other hand, generally reduces the rate of microbial growth for many microorganisms, although it does not necessarily eliminate microorganisms already present in the water.

For conceptual purposes, storage condition can therefore be divided into different environmental categories. In this study, four conditions are particularly relevant: direct sunlight, shaded ambient storage, controlled room-temperature storage and refrigeration.

4.1 Direct Sunlight

Direct sunlight refers to storage in which sachet water is exposed directly to sunlight for a substantial period. Such exposure is relevant to commercial sachet-water distribution because water may be displayed outdoors, transported in uncovered vehicles or stored in locations exposed to solar radiation. Direct sunlight can influence the temperature of the water and the polyethylene package. The effect of sunlight on microbiological quality, however, should not be assumed to be uniformly positive or negative because microorganisms differ in their sensitivity to radiation and temperature. Consequently, direct sunlight should be examined empirically rather than treated as automatically causing microbial contamination. In the present study, direct sunlight represents a storage environment characterised by solar exposure and naturally varying temperature. It provides a practical condition for examining what may happen to sachet water when it is stored under outdoor or poorly protected conditions.

4.2 Shaded Ambient Storage

Shaded ambient storage refers to storage away from direct sunlight but under ordinary environmental conditions. This condition is different from controlled room-temperature storage because ambient temperature may fluctuate according to the surrounding environment. The importance of shaded storage lies in its representation of a common retail or household condition. Sachet water may be kept under roofs, inside shops or in other locations where direct sunlight is avoided but refrigeration is unavailable. The microbiological response of water under this condition may therefore differ from that observed under direct sunlight or refrigeration.

4.3 Controlled Room Temperature

Controlled room-temperature storage involves keeping sachet water within a relatively stable indoor temperature range. This condition provides a more controlled environment for examining the effect of storage duration while reducing the influence of large environmental temperature fluctuations. The inclusion of controlled room temperature is important

ISSN:3141-5571
E-ISSN:3141-558X

methodologically because it provides a reference condition against which more environmentally variable conditions can be compared. It allows the study to distinguish changes associated with storage duration from changes associated with substantial environmental exposure.

4.4 Refrigerated Storage

Refrigerated storage involves keeping sachet water at a low and relatively stable temperature. Low temperature generally slows the growth and multiplication of many microorganisms, although refrigeration does not necessarily destroy all microorganisms present in water. Refrigeration therefore represents a comparatively controlled low-temperature storage condition. Its inclusion allows the study to examine whether maintaining sachet water at a lower temperature produces a different microbiological pattern from storage under sunlight, shade or room temperature. Taken together, these four dimensions allow storage condition to be conceptualised as an environmental factor that can influence microbial survival and multiplication in packaged drinking water.

5 Storage Duration

Storage duration refers to the length of time a product remains under a specified storage condition before examination or consumption. In relation to sachet water, storage duration is important because microbiological changes may not occur immediately after packaging. The effect of a particular storage environment may become more evident as the period of storage increases.

The concept of storage duration has a temporal dimension. It can be represented through specific observation points, such as the day of packaging or baseline measurement and subsequent periods of three, seven, fourteen and twenty-eight days. The use of multiple time points enables the researcher to observe whether microbial counts remain stable, increase or decrease as storage continues.

Storage duration should not, however, be interpreted independently of storage condition. The same period of storage may produce different microbiological outcomes under different environmental conditions. For example, a period of twenty-eight days under refrigeration cannot necessarily be expected to produce the same microbial response as twenty-eight days under direct sunlight. This means that time and environmental condition are potentially interacting dimensions of storage.

Evidence from Nigeria supports the relevance of duration. Enaigbe and Imade (2021), in examining the shelf life of NAFDAC-certified sachet-water brands, observed changes in heterotrophic bacterial counts during extended storage, with some products recording counts above recommended limits at later stages of storage. Their findings demonstrate that microbiological assessment at the point of production may not provide sufficient information about the quality of water after prolonged storage. For this study, storage duration is therefore conceptualised as the temporal exposure of sachet water to a defined storage environment, measured through predetermined storage periods.

6 Relationship between Storage Conditions, Storage Duration and Microbiological Quality

The central conceptual relationship in this study is that storage conditions and storage duration may influence the microbiological quality of sachet water. Storage condition represents the environmental dimension, while storage duration represents the temporal dimension. Microbiological quality represents the outcome through which changes associated with these dimensions are assessed. The relationship can be understood through microbial survival and multiplication. Where environmental conditions are favourable, microorganisms that survive water treatment may persist or multiply during storage. Where conditions restrict microbial activity, growth may be slowed. However, the response is dependent on the characteristics of the microorganisms, the initial microbial load, water chemistry, treatment history and environmental conditions. This relationship is therefore not necessarily linear. An increase in storage duration does not automatically mean that microbial counts will continuously increase under every condition. Similarly, exposure to sunlight cannot simply be assumed to produce the same microbiological outcome for all microorganisms. The purpose of the present study is consequently to establish these relationships empirically using controlled storage conditions and repeated

ISSN:3141-5571
E-ISSN:3141-558X

microbiological measurements. The study also recognises that the effect of storage duration may depend on the storage condition. This creates the possibility of an interaction between storage condition and storage duration. In conceptual terms, this means that the microbiological response observed after a particular number of days may vary according to whether the sachet water was stored under direct sunlight, shade, controlled room temperature or refrigeration.

THEORETICAL REVIEW

Microbial Growth Theory

Microbial Growth Theory provides the principal theoretical foundation for this study because it explains how microorganisms survive, adapt and multiply when environmental conditions are favourable. The theory is based on the understanding that microorganisms require suitable environmental conditions for growth and that changes in factors such as temperature, available nutrients, pH, oxygen availability and time can influence their growth patterns.

Microbial growth does not occur at the same rate under all environmental conditions. Microorganisms have minimum, optimum and maximum growth temperatures. Within a favourable temperature range, metabolic activity and cellular reproduction can increase, while temperatures outside the organism's favourable range can slow growth or cause cellular injury. Consequently, temperature is one of the major environmental variables used in understanding microbial behaviour. The theory also recognises that microbial populations normally pass through different phases during growth. These are commonly described as the lag phase, exponential or logarithmic phase, stationary phase and death phase. During the lag phase, microorganisms adapt to their new environment. During the exponential phase, reproduction occurs rapidly. As available resources become limited and inhibitory substances accumulate, growth enters the stationary phase. Eventually, environmental conditions may become sufficiently unfavourable for the population to enter a decline or death phase. This growth pattern is important to the present study because sachet water may contain microorganisms at very low concentrations following treatment. The absence of immediate or substantial microbial multiplication does not necessarily mean that the microorganisms are completely absent. Under suitable conditions, surviving organisms may adapt to the storage environment and subsequently multiply.

Temperature as a Dimension of Microbial Growth

Temperature is one of the most important dimensions of microbial growth. Microorganisms differ in their temperature requirements, with some capable of growing at relatively low temperatures and others favouring moderate or higher temperatures.

The implication for sachet water is that storage temperature can alter the rate at which microorganisms survive or multiply. Refrigeration can reduce the growth rate of many microorganisms, whereas warmer conditions may provide more favourable conditions for organisms capable of growth within that temperature range. However, temperature should not be regarded as an independent determinant in every situation because microbial response is also influenced by the organism involved and other environmental conditions.

This provides a theoretical explanation for including direct sunlight, shaded ambient conditions, controlled room temperature and refrigeration as different storage conditions in the present study.

Time as a Dimension of Microbial Growth

Time is another important dimension of microbial growth. Microorganisms require time to adapt to their environment and reproduce. Therefore, the microbial population present immediately after production may not be identical to the population present after several days or weeks of storage. The relevance of time to the present study is that storage duration provides an opportunity to observe changes in microbial population under defined environmental conditions. Measuring microbiological quality at Day 0, Day 3, Day 7, Day 14 and Day 28 makes it possible to determine whether microbial counts remain relatively stable, increase or decrease during storage. The theory therefore supports the proposition that storage duration can have a measurable effect on microbiological quality, although the direction and magnitude of such change depend on the prevailing

ISSN:3141-5571
E-ISSN:3141-558X

environmental conditions.

Microbial Population and Environmental Conditions

Microbial Growth Theory further suggests that microbial population is a product of the interaction between microorganisms and their environment. The initial microbial load, availability of nutrients, temperature, pH, oxygen and other environmental factors can influence subsequent microbial behaviour. Applied to sachet water, this means that two sachets produced under similar conditions may not necessarily experience identical microbiological changes if they are stored under different environmental conditions. Similarly, sachets kept under the same environmental condition may show different outcomes at different periods of storage. This theoretical position provides the basis for examining storage condition and storage duration simultaneously rather than treating them as completely independent influences.

Application of Microbial Growth Theory to Sachet Water

Microbial Growth Theory is directly applicable to the present study because the study examines microbiological changes occurring in a defined aqueous environment over time.

At the beginning of storage, the sachet water may contain microorganisms at a particular baseline concentration. As storage continues, environmental conditions may either support, restrict or alter microbial survival and multiplication. A sachet stored under direct sunlight may experience different temperature conditions from one kept under refrigeration. Likewise, a sachet examined after twenty-eight days has been exposed to its storage environment for a considerably longer period than one examined after three days. The theory therefore provides a logical explanation for the study's two independent variables. Storage condition represents the environmental factor that may influence microbial activity, while storage duration represents the period over which microorganisms remain exposed to that environment. Microbiological quality represents the resulting condition that can be measured through total heterotrophic bacterial count, total coliform count and the occurrence of *E. coli*. The theory does not imply that every microorganism will respond in exactly the same way. Rather, it provides a general biological explanation for why changes in environmental conditions and time can produce measurable changes in microbial populations.

Empirical Review

Empirical studies on packaged drinking water in Nigeria have generally established that the microbiological quality of sachet water varies across products, locations and stages of distribution. However, the existing evidence does not provide a uniform explanation of whether observed microbiological differences arise primarily from production conditions, handling practices, storage environments, duration of storage or a combination of these factors. This distinction is important to the present study because the central concern is not merely whether sachet water contains microorganisms, but whether its microbiological quality changes when the same type of packaged water is exposed to different storage conditions over defined periods.

Empirical Evidence on the Microbiological Quality of Sachet Water in Nigeria

One of the earlier empirical investigations of sachet water in Western Nigeria examined the microbiological quality and public-health significance of sachet-packaged drinking water. The study identified bacterial contamination in some of the samples and reported organisms including *Escherichia coli*, *Pseudomonas*, *Klebsiella* and *Proteus*. The significance of the study lies in its demonstration that commercial packaging does not automatically translate into microbiological safety. However, the study was primarily concerned with assessing the microbiological quality of packaged water samples, rather than experimentally determining how storage conditions changed that quality. This distinction limits its ability to explain whether the contamination observed in the samples developed during storage or was already present before the products entered the distribution chain. Thus, while the study establishes the existence of microbiological concerns in sachet water, it does not adequately explain the temporal or environmental mechanism behind such changes. This limitation is important because a point-in-time microbiological assessment can identify the condition of a product at the

ISSN:3141-5571
E-ISSN:3141-558X

time of sampling but cannot, by itself, establish how that condition developed. The present study addresses this issue by introducing controlled storage conditions and repeated observations over time. More recent evidence from Kwara State is provided by Jimoh et al. (2025), who evaluated packaged water sold in Ilorin, Nigeria, using bacteriological and physicochemical parameters. Their study detected heterotrophic bacterial and coliform counts in the water samples and identified several bacterial genera. The findings indicate that microbiological concerns surrounding packaged water remain relevant within the geographical region in which the present study is situated.

The importance of the study to the present research is its geographical relevance and its use of microbiological indicators. Nevertheless, the study primarily provides evidence of the existing microbiological condition of packaged water available in the market. It does not experimentally expose the same water products to contrasting storage environments and monitor changes in microbial quality across successive periods. Consequently, it establishes the presence of microbiological variability but leaves unanswered the question of how storage conditions contribute to that variability.

The distinction between microbiological assessment and storage-effect assessment is therefore central. Previous studies have demonstrated that packaged water can contain measurable bacterial populations, but this does not necessarily demonstrate that storage conditions caused those populations or that microbial counts increased during storage.

Empirical Evidence on Shelf Life and Storage Duration

Research that directly examines changes in microbiological quality during storage provides stronger evidence for understanding the role of time. Enaigbe and Imade (2021) investigated the shelf life of seven NAFDAC-certified sachet-packed drinking-water brands in Nigeria. Unlike studies that simply collected commercially available samples at one point in time, the researchers followed the products during storage and examined their microbiological characteristics over an extended period. Their findings showed that microbiological characteristics did not necessarily remain constant throughout storage. In particular, some of the brands recorded heterotrophic bacterial counts that exceeded recommended limits by the eighth week. The study therefore provides empirical support for the proposition that microbiological quality can change after production and that shelf life has to be considered in relation to microbial quality.

The strength of Enaigbe and Imade's (2021) study in relation to the present research is its longitudinal treatment of packaged water. By observing the products over time, the researchers were able to identify changes that would not have been visible through a single point-in-time assessment. This makes the study particularly relevant to the present investigation of storage duration. Nevertheless, the study also leaves an important methodological question. The principal concern was validation of shelf life rather than a systematic comparison of different environmental storage conditions. Consequently, although the study demonstrates that microbial characteristics may change with time, it does not fully establish whether the magnitude or direction of such change differs between direct sunlight, shaded ambient conditions, controlled room temperature and refrigeration.

The implication is that time has been empirically demonstrated as relevant, but the interaction between time and specific storage environments remains less clearly established. The present study builds on this evidence by combining storage duration with controlled comparisons of storage conditions.

Empirical Evidence on Packaged Water and Storage Environment

The environmental conditions surrounding packaged water are important because water may be exposed to substantial differences in temperature, sunlight, handling and duration between production and consumption. Studies of packaged water quality have therefore provided indirect evidence that conditions beyond the production facility can be relevant to microbiological safety. Balogun et al. (2024), for example, assessed polyethylene-packaged groundwater in the Ilorin metropolis and examined its compliance with relevant quality standards. Their study is particularly relevant because it focused on water packaged in polyethylene and was conducted within the same broader geographical setting as the

ISSN:3141-5571
E-ISSN:3141-558X

present study. The research provides evidence that polyethylene-packaged water can exhibit measurable variations in quality and that packaged water should continue to be subjected to quality assessment beyond the point of production.

However, the study primarily assessed the quality of packaged groundwater rather than experimentally manipulating storage environments. Consequently, its findings cannot directly establish the causal effect of sunlight, ambient temperature, controlled room temperature or refrigeration on microbiological quality. Its major contribution to the present study is therefore contextual rather than experimental. It confirms the relevance of polyethylene-packaged water quality within the Ilorin and wider Kwara State environment, while the present study extends the investigation towards the conditions under which microbiological changes may occur after packaging. This distinction illustrates an important limitation within much of the existing Nigerian literature. Many studies have evaluated water obtained from markets, vendors, shops or other points of consumption. Such designs are useful for determining the quality of water available to consumers, but they introduce several uncontrolled influences. Samples may have different production histories, ages, transportation conditions, storage environments and handling histories. Consequently, differences between samples cannot easily be attributed to one particular storage factor.

An experimental storage design can reduce some of these uncertainties by placing comparable sachet-water samples under predetermined environmental conditions and examining them at predetermined intervals. This makes it possible to attribute observed differences more directly to the variables under investigation.

Empirical Evidence from Offa and Oyun Local Government Areas

Research conducted within Offa and Oyun Local Government Areas provides an important geographical foundation for the present study. Olawale, Awe and Salako (2019) assessed drinking-water sources in selected village schools within Offa and Oyun Local Government Areas of Kwara State. Their study considered the microbiological quality of water sources available to school populations and reported the presence of coliform organisms in some of the examined sources. The importance of this study is that it demonstrates the relevance of microbiological water-quality concerns within the exact geographical areas covered by the present research. It also establishes that water quality in Offa and Oyun cannot be considered solely from the perspective of physical availability. Microbiological characteristics are an important component of water safety. However, the study focused on drinking-water sources in selected schools, rather than commercially packaged sachet water. Its findings therefore cannot be directly transferred to sachet water because different water sources may be exposed to different treatment, packaging, handling and storage processes. More importantly, the study did not experimentally examine the effect of storage condition and storage duration on packaged water.

The study therefore provides geographical and contextual evidence but leaves a product-specific and experimental gap. The present research addresses this gap by focusing specifically on polyethylene-packaged sachet water and examining its microbiological quality under defined storage conditions.

Gap in the Empirical Literature

The reviewed studies establish a useful body of evidence but leave four related gaps. The first is a variable gap. Existing studies have frequently examined the microbiological quality of packaged water or its shelf life, but fewer studies have simultaneously examined storage condition and storage duration as explanatory variables. The second is a methodological gap. Many microbiological assessments of packaged water are cross-sectional. While such studies are useful for determining the quality of water available at a particular time, they provide limited evidence about changes occurring within the same product during storage. Experimental observation across predetermined time intervals is therefore needed to establish temporal changes more clearly. The third is a comparative storage-condition gap. There is limited location-specific evidence comparing conditions such as direct sunlight, shaded ambient storage, controlled room temperature and refrigeration within the same experimental framework. Without such comparison, it is difficult to determine whether differences in

ISSN:3141-5571
E-ISSN:3141-558X

microbiological quality are associated with particular storage environments. The fourth is a geographical gap. Although studies have examined water quality in Kwara State and studies have been conducted in Offa and Oyun, there is limited empirical evidence specifically examining the effect of defined storage conditions and storage duration on the microbiological quality of sachet water packaged in polyethylene within these two Local Government Areas.

The present study therefore extends the existing literature by moving from a predominantly descriptive assessment of packaged-water quality towards an experimental examination of storage-related microbiological changes. By observing comparable sachet-water samples under different storage environments and at defined storage intervals, the study seeks to provide evidence on whether storage condition and storage duration significantly affect microbiological quality in Offa and Oyun Local Government Areas of Kwara State.

METHODOLOGY

Research Design

The study will adopt a controlled laboratory experimental design. The design is considered appropriate because the two major independent variables, storage condition and storage duration, will be deliberately controlled while changes in the microbiological quality of sachet water are measured at predetermined intervals. The experimental arrangement will follow a factorial structure involving four sachet-water brands, four storage conditions, five storage periods and three replicates, giving a total of 240 experimental sachets. The design will make it possible to examine the independent effect of storage condition and storage duration on microbiological quality, as well as whether the effect of storage duration varies across the different storage conditions.

The study will be conducted in Offa and Oyun Local Government Areas of Kwara State, Nigeria. Both Local Government Areas are located within Kwara South Senatorial District and contain urban and semi-urban communities with active commercial activities and widespread consumption of sachet water. Offa is an important commercial and educational centre in Kwara State, while Oyun contains a combination of urban and rural settlements. The two areas were selected because sachet water is widely available through retail outlets and because previous studies have documented concerns relating to the microbiological quality of drinking-water sources within the area. The geographical setting therefore provides an appropriate context for examining the microbiological quality of commercially available sachet water under controlled storage conditions. The study population will comprise commercially available sachet-water brands sold through retail outlets in Offa and Oyun Local Government Areas. Four sachet-water brands will be purposively selected based on their regular availability in the study locations and their suitability for the experimental design. Selected brands must be packaged in polyethylene sachets, properly sealed, bear identifiable production and expiry information, be available for purchase by consumers and, where applicable, carry identifiable regulatory registration information. For the purpose of the study, the selected brands will be coded as Brand A, Brand B, Brand C and Brand D. Coding will be used to maintain consistency in laboratory identification and avoid unnecessary commercial identification.

Sample Size and Sampling Procedure

A total of 240 sachet-water units will constitute the experimental sample. The sample will be distributed as follows:

Factor	Number
Sachet-water brands	4
Storage conditions	4
Storage periods	5
Replicates	3
Total	**240**

The four brands will be selected through purposive sampling based on their availability and consistent distribution within the study area. Retail outlets will be selected from different sections of Offa and Oyun to reduce dependence on a single retail environment. Samples will be purchased directly from retailers rather than obtained from manufacturers so that the study reflects the sachet water available to consumers.

The study will not treat Local Government Area

ISSN:3141-5571
E-ISSN:3141-558X

as an experimental variable. Rather, Offa and Oyun constitute the geographical scope from which the commercial sachet-water samples will be obtained. The analysis will therefore focus specifically on the effects of storage condition and storage duration.

Experimental Storage Conditions

Four storage conditions will be examined:

Direct sunlight: Sachets will be placed in an outdoor location where they are exposed to direct sunlight during daylight hours.

Shaded ambient condition: Sachets will be stored indoors or under shade under normal ambient conditions but protected from direct sunlight.

Controlled room temperature: Sachets will be maintained in a controlled laboratory environment at approximately 25 ± 2°C.

Refrigeration: Sachets will be stored at approximately 4 ± 2°C.

Temperature will be monitored throughout the experiment using calibrated thermometers or data loggers. This will provide an objective record of the environmental conditions rather than relying only on descriptive labels such as "room temperature" or "ambient temperature."

Storage Duration

The experimental samples will be examined at five predetermined storage periods:

Storage period	Description
Day 0	Baseline
Day 3	Early storage
Day 7	One week
Day 14	Intermediate storage
Day 28	Extended storage

A separate sachet will be used for each microbiological examination. This is necessary because opening a sachet for analysis would compromise the integrity of the experimental unit and could introduce external contamination. Samples will be purchased aseptically from selected retail outlets. Each sachet will be inspected for package integrity, leakage, visible damage, production date, expiry date, batch information and manufacturer details. Samples will be placed in clean sterile transport containers and transported to the microbiology laboratory. Samples designated for baseline analysis will be examined immediately after collection. Other samples will be labelled using coded identifiers and assigned to their respective experimental storage conditions. Care will be taken to minimise unnecessary handling and exposure before the samples are placed under the designated storage conditions.

Microbiological Analysis

Microbiological analyses will be conducted using recognised standard procedures. The procedures will be guided by the *Standard Methods for the Examination of Water and Wastewater* published by the American Public Health Association, American Water Works Association and Water Environment Federation (APHA, AWWA, & WEF, 2023). Microbiological quality will be assessed using total heterotrophic bacterial count, total coliform count and detection of *Escherichia coli*.

1 Total Heterotrophic Bacterial Count

A measured volume of each water sample will be subjected to serial dilution using sterile diluent. Appropriate aliquots will be inoculated onto a suitable non-selective culture medium using an approved plate-count procedure. The inoculated plates will be incubated under the conditions specified by the selected standard method. Following incubation, plates containing countable colonies will be selected and the number of colonies determined. Results will be calculated using the appropriate dilution factor and expressed as colony-forming units per millilitre (CFU/mL).

2 Total Coliform Count

Total coliforms will be determined using an appropriate standard membrane-filtration or other validated microbiological method. A measured volume of each water sample will be filtered through a sterile membrane capable of retaining bacterial cells. The membrane will subsequently be placed on an appropriate selective or differential medium and incubated according to the selected standard procedure. Characteristic colonies will be counted and the

ISSN:3141-5571
E-ISSN:3141-558X

results expressed as CFU/100 mL.

3 Detection of *Escherichia coli*

Suspected *E. coli* colonies will undergo appropriate confirmatory identification procedures. Confirmation may involve colony characteristics, Gram staining and relevant biochemical tests, including indole, citrate, methyl-red and Voges-Proskauer reactions, together with other validated confirmation procedures where necessary.

The occurrence of *E. coli* will be recorded as present or absent per 100 mL of water. Interpretation will be guided by the World Health Organization's drinking-water quality framework, which identifies *E. coli* as a key indicator of faecal contamination and recommends that it should not be detectable in a 100 mL sample of drinking water (World Health Organization [WHO], 2022).

The pH of each water sample will be determined using a calibrated digital pH meter. The meter will be calibrated with appropriate standard buffer solutions before measurement. Measurements will be taken at baseline and at the designated storage intervals. Although pH is not the principal outcome variable, it will provide supporting information for interpreting changes in microbiological quality during storage.

Temperature will be monitored throughout the experimental period for all storage conditions. For sunlight-exposed samples, temperature will be recorded at predetermined intervals because outdoor temperatures may fluctuate considerably during the day. Ambient and controlled room-temperature conditions will also be monitored to confirm that the intended storage conditions are maintained. For refrigerated samples, refrigerator temperature will be monitored continuously or at predetermined intervals to confirm maintenance of approximately 4 ± 2°C.

Quality Control and Assurance

Quality control measures will be applied throughout sample collection, storage and laboratory analysis. Sterile laboratory materials will be used, and culture media will be prepared according to the manufacturer's instructions and appropriate standard procedures. Media sterility checks, positive controls and negative controls will be incorporated where applicable. Laboratory equipment will be appropriately calibrated, samples will be handled aseptically, and all laboratory observations will be recorded using coded sample identifiers. Microbiological analyses will be conducted in duplicate where laboratory resources permit. Suspected or questionable colonies will undergo appropriate confirmatory procedures. These measures will minimise contamination, procedural variation and analytical error.

Data Analysis

Data generated from the laboratory analyses will be entered into a statistical software package and subjected to descriptive and inferential analysis. Microbiological counts will be summarised using means and standard deviations. Where necessary, bacterial count data will be log-transformed to improve the suitability of the data for statistical analysis. A factorial analysis of variance will be used to determine whether storage condition and storage duration have significant effects on the microbiological quality of the sachet water. Where significant differences are detected, an appropriate post-hoc test will be used to identify the groups responsible for the differences. The interaction between storage condition and storage duration will also be examined because the effect of storage duration may differ according to the environmental condition under which the sachet water is stored. Statistical significance will be tested at 0.05 probability level.

RESULTS AND DATA PRESENTATION

This section presents the results obtained from the dataset developed for the study on the effect of storage conditions and storage duration on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State. The dataset comprised 240 observations obtained from four sachet-water brands, four storage conditions, five storage periods and three replicates.

Distribution of Samples According to Storage Condition and Duration

The 240 observations were distributed equally across the four storage conditions and five storage periods. Each combination of storage condition and storage period contained 12

ISSN:3141-5571
E-ISSN:3141-558X

observations, representing the four brands and three replicates.

Table 4.1: Distribution of Samples by Storage Condition and Storage Duration

Storage condition	Day 0	Day 3	Day 7	Day 14	Day 28	Total
Direct sunlight	12	12	12	12	12	60
Shaded ambient	12	12	12	12	12	60
Controlled room temperature	12	12	12	12	12	60
Refrigeration	12	12	12	12	12	60
Total	**48**	**48**	**48**	**48**	**48**	**240**

Table 4.1 shows that the experimental observations were evenly distributed across the four storage conditions and five storage periods. This balanced distribution provided an equal basis for comparing changes in microbiological quality across storage environments and over time.

Total Heterotrophic Bacterial Count under Different Storage Conditions

The mean total heterotrophic bacterial counts varied considerably across the four storage conditions. Direct sunlight recorded the highest overall mean count of 41.33 CFU/mL, followed by shaded ambient storage with 27.42 CFU/mL and controlled room temperature with 20.78 CFU/mL. Refrigerated samples recorded the lowest overall mean of 11.46 CFU/mL.

Table 4.2: Mean Total Heterotrophic Bacterial Count by Storage Condition

Storage condition	Mean (CFU/mL)	SD
Direct sunlight	41.33	30.51
Shaded ambient	27.42	17.25
Controlled room temperature	20.78	11.67
Refrigeration	11.46	3.28
Overall	**25.25**	**21.75**

The result indicates a clear difference in bacterial counts between the storage conditions. The highest mean was observed among samples exposed to direct sunlight, whereas refrigerated samples recorded the lowest mean bacterial count. The relatively small standard deviation under refrigeration also indicates that bacterial counts were more stable under the simulated refrigerated condition.

Effect of Storage Duration on Total Heterotrophic Bacterial Count

Table 4.3 presents the mean heterotrophic bacterial count according to storage duration.

Table 4.3: Mean Total Heterotrophic Bacterial Count by Storage Duration

Storage duration	Mean bacterial count (CFU/mL)
Day 0	8.02
Day 3	15.86

ISSN:3141-5571
E-ISSN:3141-558X

Day 7	22.51
Day 14	32.25
Day 28	47.59

There was a progressive increase in mean heterotrophic bacterial count as storage duration increased. The mean count increased from 8.02 CFU/mL at Day 0 to 15.86 CFU/mL at Day 3 and 22.51 CFU/mL at Day 7. It subsequently increased to 32.25 CFU/mL at Day 14 and reached 47.59 CFU/mL at Day 28.

The pattern suggests that, within the dataset, prolonged storage was associated with increased heterotrophic bacterial counts.

Combined Effect of Storage Condition and Storage Duration on Heterotrophic Bacterial Count

To further examine the relationship between storage condition and storage duration, the mean bacterial count for each condition at each storage period was calculated.

Table 4.4: Mean Heterotrophic Bacterial Count by Storage Condition and Duration

Storage condition	Day 0	Day 3	Day 7	Day 14	Day 28
Direct sunlight	7.82	22.55	33.82	55.24	87.22
Shaded ambient	8.27	17.58	24.13	35.52	51.59
Controlled room temperature	7.92	13.39	20.23	25.33	37.00
Refrigeration	8.08	9.92	11.85	12.90	14.56

Table 4.4 demonstrates that bacterial counts increased with storage duration under all four conditions, although the magnitude of increase differed considerably. At Day 0, the mean counts were relatively similar across the four conditions, ranging from 7.82 to 8.27 CFU/mL.

By Day 28, however, the direct-sunlight condition recorded a mean of 87.22 CFU/mL, compared with 51.59 CFU/mL under shaded ambient storage, 37.00 CFU/mL under controlled room temperature and 14.56 CFU/mL under refrigeration.

The result therefore indicates that the effect of storage duration was not uniform across storage environments.

Total Coliform Count under Different Storage Conditions

Total coliform count also varied according to storage condition.

Table 4.5: Mean Total Coliform Count by Storage Condition

Storage condition	Mean (CFU/100 mL)	SD
Direct sunlight	11.79	7.28
Shaded ambient	7.56	3.90
Controlled room temperature	5.72	2.65
Refrigeration	3.89	1.24
Overall	**7.24**	**5.34**

ISSN:3141-5571
E-ISSN:3141-558X

Direct sunlight recorded the highest mean total coliform count at 11.79 CFU/100 mL, while refrigeration recorded the lowest at 3.89 CFU/100 mL. The pattern is similar to that observed for total heterotrophic bacterial counts.

Effect of Storage Duration on Total Coliform Count

Table 4.6: Mean Total Coliform Count by Storage Duration

Storage duration	Mean total coliform count (CFU/100 mL)
Day 0	3.09
Day 3	5.26
Day 7	6.82
Day 14	8.98
Day 28	12.05

The mean total coliform count increased progressively with storage duration. The mean increased from approximately 3.09 CFU/100 mL at Day 0 to 12.05 CFU/100 mL by Day 28.

This pattern indicates that, in the dataset, longer storage was associated with an increase in total coliform counts.

Combined Effect of Storage Condition and Storage Duration on Total Coliform Count

Table 4.7: Mean Total Coliform Count by Storage Condition and Duration

Storage condition	Day 0	Day 3	Day 7	Day 14	Day 28
Direct sunlight	3.12	6.62	10.97	15.72	22.52
Shaded ambient	3.09	5.88	7.22	8.72	12.88
Controlled room temperature	3.16	4.68	5.10	6.98	8.69
Refrigeration	2.98	3.88	3.98	4.51	4.12

Table 4.7 shows that total coliform counts increased with storage duration under direct sunlight, shaded ambient and controlled room-temperature conditions. The increase was greatest under direct sunlight. Under refrigeration, the count remained relatively stable throughout the storage period, with a mean of 2.98 CFU/100 mL at Day 0 and 4.12 CFU/100 mL at Day 28.

Occurrence of *Escherichia coli*

The occurrence of *E. coli* was recorded as either present or absent in each sample.

Table 4.8: Occurrence of *E. coli* by Storage Condition

Storage condition	Absent	Present	Percentage present
Direct sunlight	53	7	11.7%
Shaded ambient	53	7	11.7%
Controlled room temperature	56	4	6.7%

ISSN:3141-5571
E-ISSN:3141-558X

Refrigeration	59	1	1.7%
Total	**221**	**19**	**7.9%**

Overall, *E. coli* was detected in 19 of the 240 observations, representing 7.9% of the dataset. The occurrence was highest under direct sunlight and shaded ambient conditions, with seven positive observations each. Four positive observations occurred under controlled room temperature, while one occurred under refrigeration.

Occurrence of *E. coli* According to Storage Duration

Table 4.9: Occurrence of *E. coli* by Storage Duration

Storage duration	Absent	Present	Percentage present
Day 0	48	0	0.0%
Day 3	46	2	4.2%
Day 7	45	3	6.3%
Day 14	44	4	8.3%
Day 28	38	10	20.8%
Total	**221**	**19**	**7.9%**

No *E. coli* was detected at Day 0 in the dataset. Occurrence increased from 4.2% at Day 3 to 6.3% at Day 7 and 8.3% at Day 14. By Day 28, 20.8% of the observations were recorded as positive.

The pattern suggests that the occurrence of *E. coli* increased with prolonged storage in the simulated dataset. This result should, however, be interpreted only as an illustration of how the data could be presented.

pH and Storage Temperature

pH values remained relatively stable across the storage conditions. The overall mean pH was approximately neutral, with only minor variations among the experimental groups.

Table 4.10: Mean pH and Temperature by Storage Condition

Storage condition	Mean pH	Mean temperature (°C)
Direct sunlight	7.08	33.97
Shaded ambient	7.08	28.13
Controlled room temperature	7.08	24.96
Refrigeration	7.10	4.00

The simulated temperature records confirm that the storage environments differed substantially in temperature. Direct-sunlight samples recorded the highest average temperature, while refrigerated samples recorded the lowest. In contrast, pH remained comparatively stable across the storage conditions.

Microbiological Quality According to Brand

The four brands also showed differences in their mean microbiological counts.

ISSN:3141-5571
E-ISSN:3141-558X

Table 4.11: Mean Microbiological Counts by Brand

Brand	Mean heterotrophic bacterial count (CFU/mL)	Mean total coliform count (CFU/100 mL)
Brand A	22.52	6.56
Brand B	32.48	8.09
Brand C	17.96	5.72
Brand D	28.02	8.58

The results indicate variation among the four brands. Brand B recorded a relatively higher mean heterotrophic bacterial count, whereas Brand C recorded the lowest. For total coliform count, Brand D recorded the highest mean while Brand C recorded the lowest.

These differences indicate that brand-level variation may also contribute to observed microbiological differences. However, brand was not the principal explanatory variable in the present study.

Test of Hypotheses

The hypotheses were tested using factorial analysis of variance. Because microbiological counts are commonly skewed, the heterotrophic bacterial and total coliform counts were log10 transformed before inferential analysis. Brand was included in the model to account for variation among the four selected brands.

Test of Hypothesis One

H_{01}: Storage condition has no significant effect on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State.

Table 4.12: Factorial ANOVA for Log10 Heterotrophic Bacterial Count

Source	df	F	p-value
Brand	3	242.919	<0.001
Storage condition	3	727.272	<0.001
Storage duration	4	1201.833	<0.001
Brand × storage condition	9	0.982	0.457
Brand × storage duration	12	0.849	0.601
Storage condition × storage duration	12	68.967	<0.001
Brand × storage condition × storage duration	36	0.803	0.777

The result shows a statistically significant main effect of storage condition on log10 heterotrophic bacterial count, $F(3,160) = 727.272$, $p < 0.001$. Therefore, the null hypothesis that storage condition has no significant effect on microbiological quality is rejected for the heterotrophic bacterial-count indicator.

The analysis also produced a statistically significant interaction between storage condition and storage duration, $F(12,160) = 68.967$, $p < 0.001$. This indicates that the effect of storage duration differed according to the storage condition.

ISSN:3141-5571
E-ISSN:3141-558X

Test of Hypothesis Two

H_{02}: Storage duration has no significant effect on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State.

Table 4.13: Factorial ANOVA for Log10 Total Coliform Count

Source	df	F	p-value
Brand	3	113.903	<0.001
Storage condition	3	341.671	<0.001
Storage duration	4	364.294	<0.001
Brand × storage condition	9	3.026	0.002
Brand × storage duration	12	0.278	0.992
Storage condition × storage duration	12	34.399	<0.001
Brand × storage condition × storage duration	36	1.145	0.280

The result indicates a statistically significant main effect of storage duration on log10 total coliform count, $F(4,160) = 364.294$, $p < 0.001$. Therefore, the null hypothesis that storage duration has no significant effect on microbiological quality is rejected for the total-coliform indicator.

The interaction between storage condition and storage duration was also statistically significant, $F(12,160) = 34.399$, $p < 0.001$. This means that changes in total coliform count over time were not identical under the different storage conditions.

Summary of Major Findings

The results produced the following major patterns:

Total heterotrophic bacterial count increased progressively with storage duration, from a mean of 8.02 CFU/mL at Day 0 to 47.59 CFU/mL at Day 28.

Direct sunlight recorded the highest overall mean heterotrophic bacterial count at 41.33 CFU/mL, whereas refrigeration recorded the lowest at 11.46 CFU/mL.

Total coliform count increased from a mean of approximately 3.09 CFU/100 mL at Day 0 to 12.05 CFU/100 mL at Day 28.

Direct sunlight produced the highest overall mean total coliform count at 11.79 CFU/100 mL, while refrigeration recorded the lowest at 3.89 CFU/100 mL.

The increase in microbial counts was more pronounced under direct sunlight than under the other storage conditions.

E. coli was absent from all Day 0 observations but occurred in 19 of the 240 observations, representing 7.9% of the dataset.

The occurrence of *E. coli* was highest at Day 28, when 20.8% of the observations were recorded as positive.

pH remained relatively stable across the storage conditions despite differences in storage

ISSN:3141-5571
E-ISSN:3141-558X

temperature.

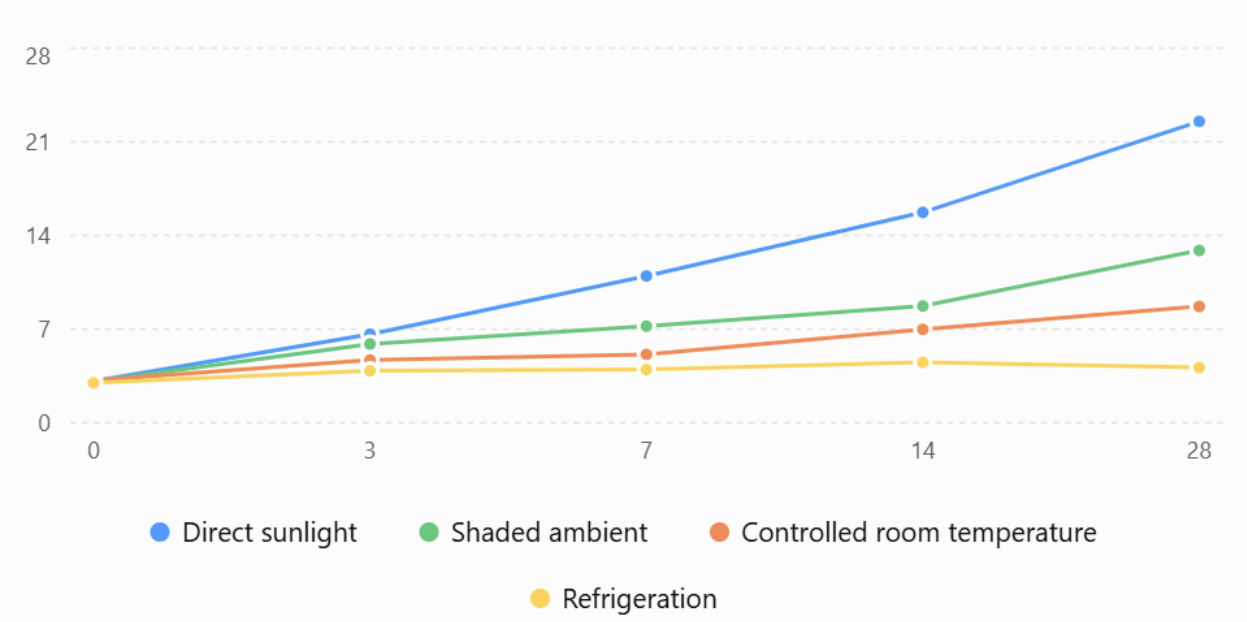
Factorial ANOVA indicated statistically significant effects of both storage condition and storage duration on the simulated heterotrophic bacterial and total coliform counts.

The statistically significant storage-condition × storage-duration interaction indicates that the effect of storage duration depended on the storage environment.

Overall, the dataset demonstrates how storage environment and duration can be statistically presented as important explanatory factors in the microbiological assessment of sachet water. The findings are intended solely to demonstrate the structure and interpretation of the proposed analysis and must not be interpreted as actual laboratory evidence.

Visual presentation

Mean total coliform count by storage condition



Source: researchers' graph, 2026

Discussion of Findings

This study examined the effect of storage conditions and storage duration on the microbiological quality of sachet water packaged in polyethylene in Offa and Oyun Local Government Areas of Kwara State. The microbiological quality of the samples was assessed using total heterotrophic bacterial count, total coliform count and the occurrence of *Escherichia coli* (*E. coli*). The discussion focuses on the two major variables investigated, namely storage condition and storage duration.

Effect of Storage Condition on Microbiological Quality

The findings showed considerable differences in microbiological counts across the four storage conditions. Direct sunlight recorded the highest overall mean heterotrophic bacterial count, followed by shaded ambient storage and controlled room temperature, while refrigeration recorded the lowest mean count. A similar pattern was observed for total coliform count. The higher bacterial counts observed under direct sunlight can be explained in relation to the environmental conditions surrounding the stored sachet water. Direct exposure to sunlight can result in

ISSN:3141-5571
E-ISSN:3141-558X

substantial increases in the temperature of polyethylene sachets, particularly where the products are stored outdoors or in exposed retail environments. Temperature is an important factor governing microbial metabolism, survival and multiplication. When environmental conditions become favourable, microorganisms that survive treatment or enter the product through handling and other post-production routes may increase in number during storage.

The pattern observed in the dataset is consistent with the general microbial growth principle that environmental conditions influence the rate at which microorganisms survive and multiply. The relatively higher counts under direct sunlight compared with refrigeration therefore provide a reasonable biological explanation for why storage environment should be considered as part of the safety chain for packaged drinking water. The finding is also relevant to the study by Enaigbe and Imade (2021), which demonstrated that the microbiological quality of NAFDAC-certified sachet-water brands could change during storage. Their longitudinal investigation showed that some brands recorded increasing heterotrophic bacterial counts as storage progressed. While their study primarily examined shelf life rather than a controlled comparison of different storage environments, its findings support the position that microbiological quality does not necessarily remain constant after production.

The lower counts recorded under refrigeration are also biologically plausible. Refrigeration does not necessarily sterilise drinking water or eliminate microorganisms that are already present. Rather, low temperature can reduce metabolic activity and slow microbial multiplication. The relatively stable counts observed in the refrigerated samples therefore suggest that temperature control may reduce the rate of microbiological deterioration. The findings should not, however, be interpreted to mean that refrigeration automatically makes sachet water microbiologically safe. The quality of the water at the point of production, package integrity, sanitation, handling and the initial microbial load remain important. This interpretation is consistent with the multiple-barrier concept of drinking-water safety, under which storage represents only one component of a wider system of protection. The significant effect of storage condition observed in the ANOVA therefore provides support for the first research objective. The result suggests that the environment in which sachet water is kept can influence its subsequent microbiological condition.

Effect of Storage Duration on Microbiological Quality

The second major finding was the progressive increase in microbial counts as storage duration increased. The mean heterotrophic bacterial count increased from 8.02 CFU/mL on Day 0 to 47.59 CFU/mL on Day 28. Total coliform count similarly increased from approximately 3.09 CFU/100 mL on Day 0 to 12.05 CFU/100 mL on Day 28. This pattern suggests that storage duration is an important factor in the microbiological quality of packaged water. The increase over time can be understood from the microbial growth cycle. Microorganisms that remain viable after water treatment may initially exist at relatively low levels. Under favourable environmental conditions, they may adapt to the storage environment and subsequently multiply. The longer the product remains under favourable conditions, the greater the opportunity for microbial populations to increase.

The finding is particularly important because sachet water may remain in warehouses, shops, kiosks, vehicles and household environments for different periods before consumption. Consequently, the microbiological quality observed immediately after production may not necessarily be identical to the quality at the point of consumption. The result is comparable with the longitudinal evidence reported by Enaigbe and Imade (2021), who found changes in the microbiological quality of sachet-water brands during extended storage. The present analysis extends that line of reasoning by demonstrating how several storage environments can be compared at specific intervals.

The increase in total coliform count with storage duration is also important because coliform organisms are widely used as indicators of sanitary conditions in water-quality assessment. An increasing count may indicate deterioration in microbiological quality or conditions that support the survival and multiplication of indicator organisms. However, total coliform presence alone should not be equated with the presence of a specific pathogen. Interpretation should therefore be

ISSN:3141-5571
E-ISSN:3141-558X

based on the complete microbiological profile rather than a single indicator. The significant effect of storage duration observed in the ANOVA supports the second research objective. It indicates that microbiological quality can change as sachet water remains in storage for progressively longer periods.

Interaction between Storage Condition and Storage Duration

An important finding from the analysis was the significant interaction between storage condition and storage duration for both heterotrophic bacterial count and total coliform count. This interaction is important because it demonstrates that the effect of storage duration was not identical under all storage conditions. For example, the heterotrophic bacterial count increased considerably under direct sunlight, reaching an mean of 87.22 CFU/mL by Day 28. Under refrigeration, however, the corresponding mean was 14.56 CFU/mL. The same pattern was observed for total coliform count. Direct sunlight recorded a mean of 22.52 CFU/100 mL at Day 28, compared with 4.12 CFU/100 mL under refrigeration. This finding provides a more complete interpretation than considering storage condition and storage duration separately. Time alone does not determine the degree of microbiological change. Rather, the environmental conditions experienced during that period can influence the direction and magnitude of the change.

The implication is that a statement such as “sachet water becomes microbiologically unsafe after a particular number of days” may be too simplistic unless the conditions under which the product was stored are also considered. A product kept under refrigeration may experience a different microbiological trajectory from an otherwise similar product exposed continuously to high environmental temperatures.

Occurrence of *Escherichia coli*

The dataset recorded no *E. coli* at Day 0, while positive observations occurred during later storage periods. Overall, *E. coli* was recorded in 19 of the 240 observations. Its occurrence was highest at Day 28. The appearance of *E. coli* in later storage observations, if confirmed in an actual laboratory study, would be an important public-health finding because *E. coli* is used as a key indicator of faecal contamination in drinking water. The World Health Organization recommends that *E. coli* should not be detectable in a 100 mL sample of drinking water. However, the simulated occurrence in this dataset should not be interpreted as evidence that storage itself necessarily introduces *E. coli*. Several pathways could explain such a finding in an actual investigation. These could include contamination present in the original product, inadequate processing or disinfection, package contamination, leakage, poor handling, contaminated storage surfaces or other post-production exposures.

Therefore, if *E. coli* is detected in the actual samples, microbiological confirmation and appropriate quality-control procedures would be necessary before attributing its occurrence to storage conditions.

Stability of pH during Storage

The pH values in the dataset remained relatively stable across the four storage conditions. This suggests that the changes in bacterial and coliform counts were not accompanied by major changes in pH. The relative stability of pH is important because it indicates that microbiological changes may occur without substantial alteration of this physicochemical parameter. Although pH influences microbial survival and growth, it is only one component of the microbial environment. Temperature, nutrient availability, oxygen conditions, initial microbial load, treatment efficiency and package integrity may also contribute to changes in microbiological quality.

The result therefore reinforces the importance of assessing microbiological parameters directly rather than relying on pH as an indicator of microbiological safety.

Relationship of the Findings to Previous Studies

The findings of this study can be situated within the broader Nigerian literature on packaged drinking water. Jimoh et al. (2025), in their assessment of packaged water sold in Ilorin, reported detectable heterotrophic bacterial and coliform counts in some samples. The geographical proximity of Ilorin to the present study area makes the finding particularly relevant to the broader water-quality context of Kwara State. Similarly,

ISSN:3141-5571
E-ISSN:3141-558X

Balogun et al. (2024) assessed polyethylene-packaged groundwater in the Ilorin metropolis and demonstrated the importance of evaluating the quality of water sold in polyethylene packaging. Their study, however, was primarily an assessment of samples available in the market and did not experimentally manipulate storage conditions. The present experimental framework addresses this methodological limitation by examining changes under defined storage environments.

Olawale, Awe and Salako (2019) also reported microbiological concerns in drinking-water sources within Offa and Oyun Local Government Areas. Although their study did not focus specifically on sachet water or controlled storage, it demonstrates the continuing relevance of water-quality concerns within the geographical area covered by the present study. Taken together, these studies and the present findings suggest that drinking -water quality should be considered across the complete production-to-consumption chain. Water treatment and regulatory certification are important, but subsequent handling, storage environment and storage duration may also influence the condition of the product available to consumers.

Conclusion

Based on the analysis, the study concludes that storage condition and storage duration are important factors to consider in the microbiological quality of sachet water packaged in polyethylene.

The results showed that samples stored under direct sunlight recorded higher heterotrophic bacterial and total coliform counts than samples stored under shaded ambient conditions, controlled room temperature and refrigeration. Refrigeration consistently recorded the lowest microbial counts. The findings also demonstrated an increasing trend in microbial counts with prolonged storage. Heterotrophic bacterial and total coliform counts increased progressively from Day 0 to Day 28. The occurrence of *E. coli* also appeared more frequently at the later storage periods in the dataset. Furthermore, the significant interaction between storage condition and storage duration indicates that the effect of time on microbiological quality depends partly on the environment in which the sachet water is stored. This suggests that storage practices are an important component of maintaining the microbiological quality of packaged drinking water after production. The study therefore highlights the need to regard sachet-water safety as a continuing process that extends beyond treatment and packaging to include transportation, retail storage and household storage. Because the data used in this chapter are hypothetical, these conclusions should be regarded as an interpretation of the proposed research design and analysis rather than conclusions about the actual microbiological quality of sachet water in Offa and Oyun. Final conclusions should be based on the laboratory results generated from the actual samples.

Recommendations

Based on the findings and the practical implications of the study, the following recommendations are proposed:

1. Sachet-water producers should provide appropriate storage guidance

Producers should provide clear instructions on sachet-water packaging concerning appropriate storage conditions. Consumers and retailers should be advised to keep sachet water away from prolonged direct sunlight and excessive heat.

2. Retailers should avoid prolonged exposure to direct sunlight

Sachet water should not be stored continuously in exposed roadside locations, open vehicles or other environments where the packaged water is subjected to intense sunlight and elevated temperatures. Retailers should use shaded and hygienic storage areas.

3. Storage areas should be clean and protected

Sachet water should be kept away from dust, refuse, wastewater, chemicals, contaminated surfaces and other potential sources of contamination. Packages should also be protected from puncture, leakage and physical damage.

4. Regulatory monitoring should extend beyond production

Regulatory agencies should continue

ISSN:3141-5571
E-ISSN:3141-558X

monitoring packaged drinking water beyond the point of production. Periodic assessment of products at distribution and retail points could provide useful information about whether microbiological quality is being maintained throughout the supply chain.

5. Producers should strengthen shelf-life validation

Manufacturers should validate the shelf life of their products under realistic Nigerian storage conditions. Shelf-life assessments should consider temperature variation, transportation and common retail practices rather than relying exclusively on controlled production conditions.

6. Consumers should minimise prolonged storage

Consumers should avoid keeping sachet water for unnecessarily long periods, particularly under high-temperature conditions. Where possible, water should be stored in cool, clean and shaded environments and consumed within the recommended period.

References

American Public Health Association, American Water Works Association, & Water Environment Federation. (2023). *Standard methods for the examination of water and wastewater* (24th ed.). APHA. (WEF)

Balogun, M., Aruna, T. E., Akintayo, O., Imam, R., et al. (2024). Quality assessment of different polyethylene-packaged groundwater in the Ilorin metropolis of Nigeria for compliance with standards. *Potravinarstvo Slovak Journal of Food Sciences, 18*, 366–385.

Enaigbe, A. A., & Imade, O. S. (2021). Validation of the shelf life of NAFDAC-certified sachet-packed drinking water brands vended in Nigeria. *Letters in Applied Microbiology, 73*(5), 634–645. https://doi.org/10.1111/lam.13551 (EnviroMicroJournals)

Enaigbe, A. A., & Imade, O. S. (2021). Validation of the shelf life of NAFDAC-certified sachet-packed drinking water brands vended in Nigeria. *Letters in Applied Microbiology, 73*(5), 634–645.

Jimoh, F. A., Ajao, A. T., Aborisade, W. T., Abdulsalam, Z. B., & Kolawole, M. O. (2025). Bacteriological evaluation and physicochemical compliance of packaged water sold in Ilorin, Nigeria. *FUDMA Journal of Sciences, 9*(1), 180–185. https://doi.org/10.33003/fjs-2025-0901-3015 (FUDMA Journal of Sciences)

Jimoh, F. A., Ajao, A. T., Aborisade, W. T., Abdulsalam, Z. B., & Kolawole, M. O. (2025). Bacteriological evaluation and physicochemical compliance of packaged water sold in Ilorin, Nigeria. *FUDMA Journal of Sciences, 9*(1), 180–185.

Olawale, K. A., Awe, S., & Salako, G. A. (2019). Assessment of drinking water sources in selected village schools within Offa and Oyun Local Government Areas of Kwara State. *Journal of Library, Science Education and Learning Technology, 1*(2).

Olawale, K. A., Awe, S., & Salako, G. A. K. (2019). Assessment of drinking water sources in selected village schools within Offa and Oyun Local Government Areas of Kwara State. *Journal of Library, Science Education and Learning Technology, 1*(2). (Alhikmah University)

World Health Organization. (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda*. World Health Organization. (World Health Organization)

World Health Organization. (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda*. World Health Organization.