**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,**

**KUMASI**

**COLLEGE OF SCIENCE**

**FACULTY OF BIOSCIENCES**

**DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY**

# BARRIER POTENTIAL OF PLASTICS USED FOR PACKAGING WATER (SACHET), TO MICROBIAL GROWTH AND SURVIVAL

**By**

**ZETA KOMLA**

**(BSc (Hons) Nutrition and Food Science)**

**September, 2016**

**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,**

**KUMASI, GHANA**

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**A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF**

**MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT**

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## DECLARATION

I hereby declare that this dissertation is produced out of my own work towards the award of an MSc. degree, and that to the best of my knowledge, it contains no material previously published by another person nor material which has been offered for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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| (Head of Department) | Signature | Date |

## DEDICATION

To the Almighty God and The Lord Jesus Christ for the mercies and grace shown me that enabled me to see this work to completion.

And

To my daughter Diamond Aviela Talata Elinam Nabil, and my son Jasper Aviel Nabil. You have all been a source of inspiration during difficult times of my studies.

Love you all.

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## ABSTRACT

Packaging, the technology of enclosing or protecting products for distribution, storage, sale, and use, is gaining growth in the food industry in Ghana. Drinking water, for instance, is packaged in plastic sachet or plastic bottles with Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) being the two kinds of polyethylene plastics used for the packaging of sachet water. This study evaluated the barrier potentials of these polyethylene's, used in packaging sachet water, to microbial growth and survival. A water packaging company was contracted to package treated water from one source and of same quality into the LDPE and HDPE packaging types. They were stored under three storage conditions (refrigeration, room temperature, and sunlight) for a period of six (6) weeks. It was observed that, pH of the samples decreased insignificantly for both packaging types ($p>0.05$) at the end of the investigation. For the Low Density Polyethylene (LDPE), the Total Plate Count (TPC) in the LDR (low density polyethylene under room temperature storage) increased from log 2.22 cfu/ml to log 2.33 cfu/ml while that for the samples in LDF (low density polyethylene under refrigeration), increased from log 2.22 cfu/ml to log 2.54 cfu/ml. The TPC for samples from the LDS (low density polyethylene under sunlight exposure), decreased from log 2.22 cfu/ml to 1.80 cfu/ml. For the High Density Polyethylene (HDPE), the TPC for the samples from the HDR (high density polyethylene under room temperature) increased from log 2.22 cfu/ml to log 2.98 cfu/ml whilst a decrease from log 2.22 cfu/ml to log 2.12 cfu/ml was observed in the samples from the HDF (high density polyethylene under refrigeration). For the samples from the HDS (high density polyethylene under sunlight exposure), there was a decrease from log 2.22 cfu/ml to log 0.57 cfu/ml. Generally, it was observed that increase in temperature resulted in significant increase in TPC ($p<0.05$). Coliforms were not detected in any of the samples investigated. The study revealed that, HDPEs used for packaging sachet water provided better barrier to microbial contaminants than the LDPEs.

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# CHAPTER ONE

## 1.0. INTRODUCTION

Packaging can be described as the discipline, ability and skill to enclose or protect products for delivery to customers, storage, sale and use. Additionally, it is the process of design, evaluation, and production of packages. Designing and manufacturing of packaging materials is a multi-processing step that involves careful and numerous considerations to successfully engineer. The final package will have to possess required properties in relation to its use; such as, maintenance of the safety of the product, extension of shelf-life, cost-efficiency, ecofriendly, and consumer handiness.

In this modern society, the compositions of variety of materials used are made from plastics. Rubber or plastics has become part of human life in one form or another. They are used in public health sector, clothing and footwear as well as products for use in food industry (Marsh and Bugusu, 2007).

The applications of plastics in packaging food and water play a role in the safety and health of consumers; for instance, they help in the storage and supply of clean drinking water in areas with water problems. Plastics used in the packaging of food permit the safe storage of fresh food produce as well as other foods. This is made possible by the use of atmospheric temperature control technology in the packaging, thus the use of oxygen scavenger and gas flush technology (Andrady and Neal, 2009).

In a news discussion on plastics with Steve Russell (http://www.plasticsnews.com/article/20150323/multimedia01/150329906/conversations-with-plastics-news-steve-russell-acc), he indicated that plastic packaging is able to meet the diversity of consumer needs due to their adaptability, varying types and exceptional properties. Choices such as, colour, weight, size, shape, utility, printing, and protection among many others, made along the way are met. Plastic packagings have arrows and numbers, which serve

as their codes of identification. Lots of consumers are conversant with these codes. The identification codes signify the type of polymer or plastic resin used in the manufacture of the package. The plastic resin actually comes from recycling but helps in recognizing the common plastic type used for packaging [high density polyethylene (HDPE), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polypropylene (PP), low density polyethylene (LDPE)] as well as other uses. Each resin type has specific properties making it more or less appropriate for the different packaging types (Russell, 2015).

Food package is often seen simply as a means of convenience to convey food product from place of manufacture to where it is needed; however, critical observation into the types of packaging materials being used (examples, retail stores, market place, mini shops) and the huge range of different packaging types that have been developed, widely informs us of the different uses and/or importance of food package. For instance, shaped and/or coloured packs help in product marketing while sealed packs prevent cross contamination with foreign bodies and micro-organisms. Packaging with particular barrier properties also maintains certain gas atmospheres extending the life of food with high temperature resistant packs allowing for thermal processing, such as, pasteurization or sterilization of foods, thus, increasing shelf-life and reducing food safety risks; among others (http://www.thermoscientific.com).

Maxcy-Rosenau-Last (1998), as cited in Yousaf and Chaudhry (2013), defined healthy and safe water as "that which is not contaminated with harmful or deadly agents, pleasing to taste and smell and free from dangerous chemical substances". Water suitable for drinking should be clean and be able to purify, revive, nurture, cure and revitalize the body entirely and such water is referred to as the living water (Mendie, 2001 as cited in Jeje and Oladepo, 2012).

According to Fawell and Nieuwenhuijsem (2003), contaminants naturally exist in all water especially, contaminant by inorganic source and by both microbial and chemical sources. National Standards or International guidelines of which the most significant are the WHO

Guidelines for Drinking-Water Quality ascertain the safety of drinking water (Fawell and Nieuwenhuijsen, 2003). Documents that describe lots of safety aspects of water are used as a support to regularly revise these guidelines. It has been stated that the existence of microorganisms in a product does not mean the product is hazardous for consumption or the quality is substandard (Working Document on Microbial Contaminant Limit for Microbial Pest Control Products, 2012). Food and water are mostly contaminated with bacteria, yeast and moulds, except they are pasteurized or treated. These microorganisms can be harmless or harmful by causing spoilage or disease. When hygiene or sanitation is compromised, the likelihood of products being considered as a hazard to consumers becomes increasingly significant. Numerous organizations recognized internationally; such as the Joint FAO/WHO Codex Alimentarius Commission and the International Commission on Microbiological Specifications for Foods (ICMSF) as well as regional/country jurisdictions (European Commission Regulation [EC] No. 2073/2005) have, thus, developed sampling plans, hygienic practices and microbiological specifications as well as additional composite programs such as the Hazard Analysis Critical Control Point System (HACCP) to aid in preventing diseases originated from water and food. It is, therefore, important that after water is treated for use, handling, storage and distribution are taken into high consideration to prevent re-introduction of these microorganisms.

In Ghana, microbial quality of packaged water registered by the Food and Drugs Authority, pass through specified analyses including microbial and physicochemicals.

## 1.1. PROBLEM STATEMENT

Badriah *et al*. (2012), studied the "Effect of Packaging Materials on the Physico-Chemical, Microbiological and Sensory Quality of Cooked Oggtt (dried fermented dairy product made from goat, camel or sheep milk)" where the cooked Oggtt was packaged LDPE, HDPE and

PET. They found out that, packaging significantly ($p \leq 0.05$) affected the moisture content, titratable acidity, microbiological and sensory qualities of cooked oggtt during storage for 60 days. Ajala *et al*. (2011), also worked on the "Effects of Different Packaging Materials on Bacteriological Quality of 'Egidi' (also called 'Kati', a ready to eat traditional cereal based food found in the south west region of Nigeria) with storage time". The food was packaged in nylon, aluminium foil and banana leaves and stored at ambient condition for 3 weeks. Their results showed that the initial bacterial load of the Egidi was 23 x 10 cfu/g. The total bacterial count ranged from 33 to134 x 10 cfu/g for 'Egidi' packed with nylon, 28 x 10 cfu/g for 'egidi' packed in aluminium foil and 39 to 168 x 10 cfu/g for 'Egidi' packed in banana leaves.

For more than 40 years, it has been established that few days after the production and filling of non-carbonated natural mineral water, growth of bacteria occurs during room temperature storage (Loy *et al*., 2005).

Unfortunately, packaging material used for packaging treated water has not been considered as a contributing factor to the bacterial growth and this study, thus, focused on the barrier potential of plastics used for packaging water (sachet) to the growth of microbes.

## 1.2. JUSTIFICATION

It is hoped that data from this study will reveal the effect of the two plastic packaging materials to microbial stability of packaged water (sachet) under different storage conditions with time. Information from this study is expected to help establish the right plastic packaging material to be used by industries in the water business to package sachet water, especially in Ghana.

## 1.3. MAIN OBJECTIVE

To evaluate the barrier potential of plastics, used to package (sachet) water, to microbial growth and survival.

## 1.4. SPECIFIC OBJECTIVES

- ✓ To determine the pH and temperature of the sachet water in the two different plastics used for the packaging under varying conditions of storage and length of time.
- ✓ To assess the microbial contamination of the sachet water in the two different plastics used for the packaging under varying conditions of storage and length of time.
- ✓ To compare the different parameters (pH, temperature, and microbial) between the different packaging types for the water (sachet) with varying storage time and conditions.

# CHAPTER TWO

## 2.0. LITERATURE REVIEW

## 2.1. THE IMPORTANCE OF FOOD PACKAGING

Packaging of food offers many benefits and advancement in food processing technology and has a corresponding advancement in the packaging technology industry. Packaging helps to maintain the integrity of foods after manufacturing, allowing products to be moved safely for distances from manufacturing plants to the time of consumption without changes in their wholesomeness.

Protection of the food product against damage and external contamination, food containment and provision of information on ingredients and nutrition to consumers are the main functions of packaging food (Coles 2003 as cited in Marsh and Bugusu, 2007). Secondary functions of food packaging of increasing significance are traceability, handiness, and tamper indication. The core purpose of packaging food is to economically hold the product in a way that will fulfill the requirements of the industry as well as the desires of consumers, retain the safety of the food, and reduce impact on the environment.

### 2.1.1. Protection and Preservation

Food product must be protected against being dropped, crushed, and also from the vibrations it suffers during transportation (Ryan, 2011). According to Marsh and Bugusu (2007), food packaging can help retain valuable effects of manufacturing, preserve or increase the safety and quality of food, prolong shelf-stability and retard deterioration of product. Hence, three (3) main types of external influences are protected against by packaging; chemical influence, biological influence, and physical influence.

Firstly, chemical defense reduces variations in the composition of food caused by environmental influences such as increase or decrease in moisture, exposure to gases

(especially oxygen), or light (whether visible, ultraviolet, or infrared). Some fabrics that are used in packaging can provide chemical barriers. For Instance, metals and glass offer a virtually complete barrier to penetration to chemical and other external agents. Generally, metal and glass are less permeable compared to plastics, but plastic packaging materials provide a broader range of barrier properties than those made up of metal or glass (www.ift.org/knowledge-centre).

Secondly, biological influences also affect the biological composition of food. Packaging provides biological protection as barriers to microorganisms thus help in the prevention of disease and spoilage. Again, biological barriers ensure that the right conditions prevail to control senescence (ripening and aging). A variety of mechanisms are employed to ensure such barriers function properly. These include prevention of odour transmission; maintenance of the internal environment of the package and accessibility of the product is also prevented (www.ift.org/knowledge-centre).

Finally, packaging ensures physical protection against physical influences. Physical protection serves to protect food from mechanical damage that might occur during distribution when the products are being transported. Usually, physical barriers are developed from corrugated materials and paperboard to resist crushing damage abrasions, and impacts. They are commonly used to package food that are easily perishable and subtle, example fresh fruits and eggs and as vessels in shipping. Also, plastic packaging materials are used instead of glass for frequently used products such as soft drinks (coke and soda) and shampoos to minimize the hazards from broken glass containers (www.ift.org/knowledge-centre).

According to Brunazzi *et al*. (2014), packaging is associated with the ways of safeguarding the entire integrity of a food product at the stages of transfer and storage periods. Generally, protection needs essentially the defense of the integrity of the food product against certain environmental physical causes, such as, ultraviolet (UV) radiations, crashes, powders, thermal

leaps, compression, and environmental chemical causes such as, moisture, deadly or harmful materials; and other chemical pollutants among others.

Either of the above agents or all can damage the integrated food product when they come into contact with the food products. The damage caused to the food product can result in hygienic problems and/or simple degrading failures. Thus, naturally the food package is the first and most exposed barrier for the food.

Brunazzi *et al*. (2014) further explained that protection of food is associated with ways of defense of the food product from intrinsic and extrinsic agents that could enhance the degradation of the food product.

The package in which the food is contained should 'transfer' features that are peculiar to the packaged product (Brunazzi *et al*., 2014).

### 2.1.2. Containment and Waste Reduction

There have been numerous reports on food wastage and one of the technical purposes of packaging is to contain foods (holds the content, keep it secure, clean with no leakage or breakage). Packaging helps reduce food wastage through the entire food supply chain. According to FAO report in 1989 (as cited in Marsh and Bugusu, 2007), major food wastage was reported in many countries in the world: food grain - 25% to fruits and vegetables - 50%. Due to inadequate processing/preservation methods for food produce and lack of good transportation systems, there has been high incidence of postharvest losses in the food industry and the world at large (Opara and Mditshwa, 2013

### 2.1.3. Marketing

Packages, by their design, may enhance or improve product appearance and/or to distinguish it from the competitive brands (Marsh and Bugusu, 2007). Packaging of a product becomes its

face to the purchaser. More often than not the package is the only basis of exposure to the product that consumers experience prior to purchase. Therefore, in marketing, it has become imperative for packaging to be distinctive and innovative in order to boost sales in a competitive business environment.

### 2.1.4. Product Identification and Labeling

Packaging helps in product identification and gives instructions for use, handling and storage (www.practicalaction.org) and also provides surface for labeling. Labels provide information on the product to the purchaser such as pricing, brand identification, and cooking instructions. Package label satisfies legal requirements such as manufacturer information, dates of manufacture and expiry of product, ingredient declaration, nutritional value, and net weight or volume. Larger labels accommodate recipes (Marsh and Bugusu, 2007).

### 2.1.5. Traceability

Traceability is defined as "the ability to follow the movement of a food through specified stage(s) of production, processing and distribution" (Codex Alimentarius Commission 2004 as cited in Doyran, 2009). Three (3) objectives of traceability are: to ease trace-back for the purposes of food quality and safety, to improve management in supply and to separate and sell foods with undetectable or subtle quality features (Golan and others 2004 as cited in Marsh and Bugusu, 2007). Marsh and Bugusu (2007), stated that companies that manufacture food integrate these distinctive codes onto products packaging labels for traceability purposes (allowing the manufacturers to trail their products through-out the delivery chain). The unique codes are batch numbers or code numbers or lot numbers. According to Vats (2013), the codes are a mark of identification by which foods can be traced in manufacturing and identified in distribution, and it is usually given on the label of the package.

### 2.1.6. Convenience

Features of packaging give convenience to handle through the production process, storage and distribution systems, as well as aid in product visibility, accessibility, dispensing, resealability, and being suitable for easy disposal, recycling or re-use (www.practicalaction.org; Marsh and Bugusu, 2007).

### 2.1.7. Tamper Indication

Marsh and Bugusu (2007), argued that designs of packaging both in the food and drugs industries help to lessen or eradicate the danger of adulteration and tampering. Though any package can be breeched, features that are tamper-evident cannot be re-placed easily. Tamper-evident qualities comprise special membranes, banding, breakaway closures, and special printing on bottle liners or composite cans such as graphics or text that irreversibly change upon opening.

### 2.1.8. Other Functions

According to Marsh and Bugusu (2007), packaging performs other roles, such as a carrier for rewards or bonuses (for instance addition of gifts, souvenirs) or bowls for household use. The possibility of packages being used and/or reused reduces its entry to the waste stream.

### 2.1.9. Summary of the Importance of Packaging

Packaging has evolved and this has widened its purposes from containing products to newer roles such as trailing and tracing, indicating the shelf-life of the product within the package to the consumer, directions of use, among others. The increasing dynamics in consumer demands and expectation of packaging, functions and roles of packaging can be categorized into various functions as found in Table 2.1:

**Table 2.1 Various Roles of Packages**

| Protective Function | Convenient Function | Psychological Function | Graphic Function |
|---|---|---|---|
| ✓ Vibration<br>✓ Shock/Drop<br>✓ Pressure<br>✓ Heat<br>✓ Water or moisture | ✓ Transportation<br>✓ Stacking during storage (user-end, warehouse)<br>✓ Size protection<br>✓ After re-use productivity | ✓ Attraction<br>✓ Provision of information | ✓ Design<br>✓ Colour<br>✓ Image |

Source: ONICRA (2014)

## 2.2. TYPES OF PACKAGING MATERIALS FOR FOOD

According to Robertson (2011) as cited in Opara and Mditshwa (2013), in the food industry, from farm to fork, different material types used for packaging and formats of packaging are used in the handling, storage and distribution of both fresh and processed food products. Different packaging materials have variety of performance features that exert important impacts on the shelf life of the food products. Therefore, the nature of the food product determines the type of packaging materials used in the packaging. These different packaging containers and/or materials are made from materials such as glass, plastic, metal, cardboard. Robertson (2010), as cited in Opara and Mditshwa (2013), also asserted that food products in liquid form are usually packaged in bottles and glass jars, and food products in the solid form are mainly packed on plastics and cardboards. Processed fruit and vegetables, to inhibit transmission of oxygen that can cause products spoilage, are mostly packed in airtight metal containers. The spoilage of the product can be through lipid oxidation and growth of microorganisms. According to the World Packaging Organization , as cited in Opara and Mditshwa (2013), the most important consumer packages are made of paper and board (38%), followed by plastics (30%) with rigid plastics alone taking an 18% share, metal (19%), glass (8%), and others (5%). Paperboard forms 48% of 70% (approximate) of total consumer packaging used in the food industry. Table 2.2 shows Food Packaging Types and some uses.

**Table 2.2. Food Packaging Types and Example of their uses**

| Firm Type of Packaging | Semi-firm Types of Packaging | Flexible Types of Packaging |
|---|---|---|
| ✓ Bottles made from glass<br>✓ Metallic box<br>✓ Metal cans<br>✓ Boxes made from wood | ✓ Bottles made from plastic<br>✓ Boxes made from paper carton | ✓ Plastics, Paper,<br>✓ Cellophane<br>✓ Film, Aluminium foil |

(Courtesy: ONICRA, 2014)

### 2.2.1. Food Product Types and Type of Packaging

Table 2.3 shows everyday formats of packaging different food products.

**Table 2.3. Everyday formats of packaging different products**

| **Packaging Format** | **Example of Produce** |
|---|---|
| Paperboard cartons | Fresh produce (strawberry) |
| Polyethylene-laminated cartons | Processed produce (mango juice) |
| Wooden Box | Fresh produce (strawberry) |
| Tetra recart carton | Processed produce (meat) |
| Tetra wedge package | Manufactured produce (meat) |
| Can | Manufactured food (slightly processed tomato pulp) |
| Glass bottle | Slightly processed food (pineapple juice) |
| Plastic bottle | Manufactured food (citrus juice) |

[Source: Opara and Mditshwa (2013)]

## 2.3. PLASTIC FOOD PACKAGING MATERIALS AND ASSOCIATED FOOD SAFETY RISKS

Literature makes it known that many plastic materials are commercially available; however, few of these succeed to be part of the commodity thermoplastics with respect to their high volume and relatively low price. These plastics in their fractional consumption on a global basis are shown in Figure 2.1. Low-Density Polyethylene (LDPE), Polyvinyl chloride (PVC), High-Density Polyethylene (HDPE), Polystyrene (PS), Polypropylene (PP) and Polyethylene terephthalate (PET) make up of nearly ninety percent (90%) of the global total demand (Andrady and Neal, 2009).

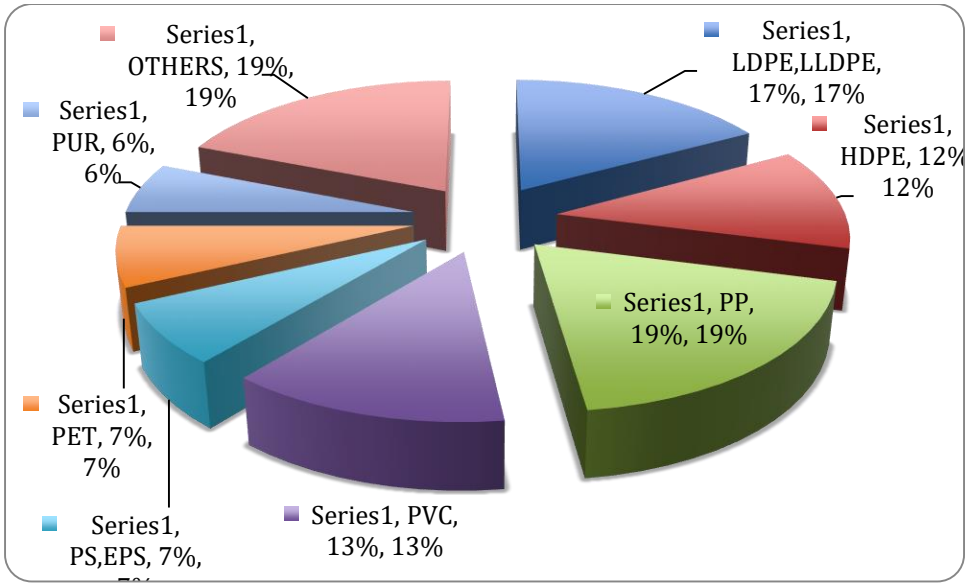


**Figure 2.1. Plastic Materials as Demanded By Resin Types Worldwide**

(Source: Andrady and Neal, 2009).

According to Brunazzi *et al*. (2014), the different types of polyethylene (PE) available are dependent on the density of the polymeric materials produced. Under high pressures and temperatures, ethylene is polymerize to produce plastic (Figure 2.2).

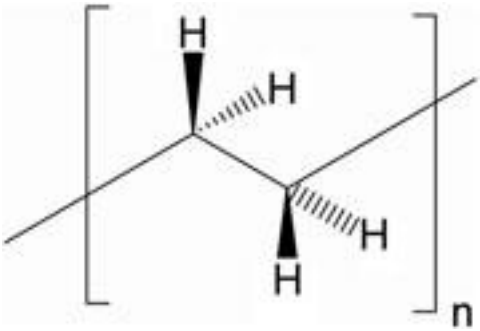


**Figure 2.2 A Simplified Chemical Structure of Polyethylene Chains**

(Source: Brunazzi *et al*., (2014)

The compactness of produced materials is determined at the polymerization stage and it is dependent on pressure, catalyzers and temperature. Hence, the decrees of polymerization may differ with the subsequent reduction of 'empty spaces' in the polyethylene's tri-dimensional

matrix (as shown in figure 2.2 above) [Monagas, *et al*., (2004) as cited in Brunazzi *et al.*, (2014)].

Polyethylene products are identified as low, medium, very low, linear medium, linear low, and high-density resins. Low-density polyethylene (LDPE) resins are used in the making of cable insulation, squeezable bottles, and grocery bags. Very low-density polyethylene (VLDPE) resins are used in the manufacture of heat sealed films and pouches and boil-in food packages. Linear low-density polyethylene (LLDPE), medium-density polyethylene (MDPE) and linear medium-density polyethylene (LMDPE) resins are used in the production of overwrap film, flexible food packaging, stretch film and shrink-wrap. High-density polyethylene(s) (HDPE) resins are used in the production of pressure pipes, children's play toys, and industrial drums. According to Andrady and Neal (2009), polyethylene is the second most used class of resins globally. There are diverse grades of PE categorized based on the average density of the resin and these are as follows: Linear LDPE (LLDPE) - 0.925 g/cm$^3$, LDPE, 0.930– 0.935 g/cm$^3$, Medium density polyethylene (MDPE), 0.93–0.945 g/cm$^3$, and HDPE, 0.945–0.965 g/cm$^3$.

Safe food is a concern to manufacturers and consumers as a whole. Food safety cannot be achieved without paying particular attention to packaging, that is, the safety of the packaging material to be used in packaging the final product. The vital role of packaging food is to ensure that the safety of finished product is secured for consumption; however, this aspect can be missing in the food chain if the packaging material does not have the right properties. As stated earlier, Russell (2015), indicated that plastic packagings have arrows and numbers, which serve as their codes of identification. The identification codes signify the type of polymer or plastic resin used in the manufacture of the packaging and the health risk associated with their uses. Table 2.4 shows type of plastics, their uses and associated health risk.

**Table 2.4. Plastics and their uses with Associated Risk**

| PI Code | Type of Plastic | Uses | Risk |
|---|---|---|---|
| 1 | Polyethylene terephthalate (PET) | Drinking bottles, jars and tubs, and snack wrappers | Do not leach any harmful/lethal chemicals. But not safe for continual use. |
| 2 | High density polyethylene (HDPE) | Toys, bottles, utensils, manufacturing equipment films and pipes. Cables and wires insulations | Do not release any harmful/deadly chemicals |
| 3 | Polyvinyl chloride (PVC) | Extruded wires covering toys, door and window components, pipes, film and fabric coatings and bottles, | PVC usually contains phthalates, which are endocrine disruptors –and suspected to be human carcinogen. Not safe, should be avoided. |
| 4 | Low density polyethylene (LDPE) | Toys, utensils, films, bottles, pipe and processing equipment. Wire and cable insulations | Do not leach any harmful/lethal substances |
| 5 | Polypropylene (PP) | Food pouches and bags. Often metallized and printed | Do not leach any harmful/lethal substances |
| 6 | Polystyrene (PS) | Some cosmetic containers, jewel boxes | Can leach butadiene and styrene that are suspected to be carcinogenic to humans. Not safe, should be avoided |
| 7 | The number 7 is used to represent any other plastics, [polycarbonate, polyurethanes (PUR)]. | Medical devices | BPA is an endocrine disruptor. Bottles and vessels made from Polycarbonate release this Bisphenol A (BPA) into liquid stored in them. Not safe, should be avoided |

PI Code = Plastic Identification Code; (Source: Barrett, 2013)

Food manufacturers consider several factors before making a choice on the type of packaging material to use in packaging the final product (ONICRA, 2014). These factors are; the cost of packaging and the functionability of packaging.

### 2.3.1. Plastic packaging types and examples of packaging material

**Plate 2.1. PET Packaging Materials**


**Plate 2.2. HDPE Packaging Materials**

**Plate 2.3. Materials made of PVC**


**Plate 2.4. LDPE Packaging Materials**


**Plate 2.5. PP Packaging Materials**


**Plate 2.6. PS Packaging Materials**

**Plate 2.7. Polycarbonate Packaging Materials**

## 2.4. POLYETHYLENE PACKAGING EFFECT ON THE QUALITY OF FOOD WITH STORAGE TIME

Works has been carried out on the comparative effect of using Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) packaging on the quality of food with storage time.

Garima and Anand (2014), investigated the "Effect of LDPE and HDPE on Storage Stability of Weaning Food prepared from Banana, Pineapple Pomace and Pulse" and showed that there was decrease in ascorbic acid, protein, and ash content of the product packaged in laminated LDPE and HDPE with increasing storage time. The HDPE was, however, of superior packaging compared with LDPE as the loss of nutrient was insignificant in weaning food packaged in HDPE material.

Daramola *et al*. (2010), worked on the "Effects of Packaging Material on the Quality of 'Pupuru' flour (a fermented cassava product dried by smoking) with storage time". The flour was packed in PVC, LDPE, HDPE and PP, and uncovered PVC as control stored at ambient condition for 24 weeks. Their results showed that packaging insignificantly influenced the chemical, functional, microbiological and sensory quality of the stored flour as well as the shelf

life. They also found out that flour samples packed in HDPE gave the highest estimated shelf life and was therefore considered the best packaging material for the 'pupuru' flour.

Badriah *et al*. (2012), also studied the "Effect of Packaging Materials on the Physico-Chemical, Microbiological and Sensory Quality of Cooked 'Oggtt' (dried fermented dairy product made from goat, camel or sheep milk)" packaged into LDPE, HDPE and PET. They found out that packaging significantly ($p \leq 0.05$) affected the moisture content, titratable acidity, microbiological and sensory qualities of cooked 'oggtt' during storage of 60 days.

Packaging materials effects and storing times on the qualities of degermed and whole maize flours were biochemically investigated by Pradyuman *et al*., (2013). The flours were packed in aluminium-laminated foil (ALF), low density polyethylene (LDPE) and high density polyethylene (HDPE) packages and the biochemical qualities were investigated during a storage period of 70 days. They concluded that maize flour could be best kept in ALF packages followed by HDPE and LDPE packages.

Eke *et al*. (2012), investigated the quality of Dambu-nama, (a Nigerian traditionally spiced, cooked, pounded, shredded and dried meat product) in different packaging materials: LDPE, HDPE, aluminium foil and plastic containers. The samples were stored for sixteen (16) weeks under room temperature. The results revealed that although growths of microorganisms in the samples were insignificant, there were gradual decreases in pH in all the samples in the different packaging materials during the storage time.

Ajala *et al*. (2010), also worked on the "Effects of Different Packaging Materials on Bacteriological Quality of 'Egidi' also called 'Kati' (a ready to eat traditional cereal based food found in the south west region of Nigeria) with storage time". The food was packaged in nylon, aluminium foil and banana leaves and stored at ambient condition for 3 weeks. Their results showed that the initial bacterial load of the Egidi was 23 x 10 cfu/g. The total bacterial count

ranged from 33 to134 x 10 cfu/g for ‘Egidi’ packed with nylon, 28 x 10 cfu/g for ‘egidi’ packed in aluminium foil and 39 to 168 x 10 cfu/g for ‘Egidi’ packed in banana leaves .

## 2.5. PLASTICS USED FOR PACKAGING WATER IN GHANA

An interview with a plastic manufacturing company in Accra, Ghana, revealed that Polyethylene terephthalate (PET) is the major plastic material used for packaging bottled water. The plastic raw materials used for packaging water (sachet) are basically of two different kinds, that is, the High Density Polyethylene (HDPE) and the Low Density Polyethylene (LDPE). The High Density Polyethylene (HDPE) is a blend of 70% high density polyethylene (HDPE) and 30% linear low density polyethylene (LLDPE) while the Low Density Polyethylene (LDPE) is a blend of 70% low density polyethylene and 30% linear low density polyethylene. The plastic materials have different properties for which a manufacturer may choose one over the other.

### 2.5.1. Manufacturing process, advantages and disadvantages of PET

PET or PETE (Polyethylene terephthalate) is a polyester and comprises of polymerized monomer ethylene terephthalate units, with repeating $C_{10}H_8O_4$ units. PETE is normally recycled.

The thermal and processing history of polyethylene terephthalate determines its state. That is if it should be both as semi-crystalline and amorphous (transparent) polymers. The semi-crystalline material may appear either white and opaque (with particle size of little micrometers) or transparent (particle size <500mm) and it is dependent on the particle size and crystal structure. Its monomer Bis (2-hydroxyethyle) terephalate can be synthesized through the esterification reaction with water as a by-product. Polymerization is by a reaction of the monomers in a process called polycondenzation.

The main advantages include the fact that they are of high quality, are easy to recycle and are of food grade. Thus, they are very suitable for drinks and food. Disadvantages include the fact that greatest numbers of plastics are made from fossil fuel oil, which is depleting in nature; they are not degradable in the soil and hence have to be recycled. They do break down into smaller sizes that may be unsafe to animals and also where they float in the oceans they are eaten by sea birds and animals, often causing their deaths.

### 2.5.2. Manufacturing process, advantages, and disadvantage of LDPE

LDPE (Low Density Polyethylene) at room temperature is not reactive. A density range of 0.910–0.940 g/cm$^3$ defines LDPE. Room temperatures range between 20 and 26 $^o$C (68 and 79 $^o$F), with an average of 23 °C (73 °F). However, in some solvents and strong oxidizing agents, LDPE can bulge. It can tolerate temperatures of 80 °C continuously and 95 °C for a short period. It is made in opaque or translucent variants, it is quite tough and flexible but can be brittle (www.calpaclab.com/reference-chart-temperature).

LDPE is more branched (on about 2% of the carbon atoms) compared to HDPE. Hence, its intermolecular forces are weaker, has lower tensile strength, and higher resilience. The molecules are less crystalline and less tightly packed due to the side branches, resulting in a lesser density. LDPE comprises the chemical elements such as hydrogen and carbon. Advantages of LDPE include the fact that they are inexpensive, impact resistant from -40 $^o$C to 90 $^o$C, are moisture resistant with good resistance to chemicals, and easily processed by all thermoplastic techniques. Poor resistance to weathering, stress cracking, high thermal expansion, difficult to bond, flammable, poor temperature capability, poor resistance to UV, and high gas permeability (especially $CO_2$) are some of the disadvantages (www.calpaclab.com/reference-chart-temperature).

### 2.5.3. Manufacturing process, advantages, and disadvantages of HDPE

HDPE (High Density Polyethylene) is a linear polymer manufactured by catalytic method from ethylene. Due to its non-branched nature it has more intently packed structure with a greater density and fairly higher resistance to chemicals compared to LDPE. High Density Polyethylene is also more opaque and slightly harder and it can withstand relatively higher temperatures (110 °C continuously and 120 °C for short periods). HDPE lends itself particularly well to blow molding.

Resistance to moisture, low cost, good resistance to chemicals, impact resistant from -40 $^{o}$C to 90 $^{o}$C, and readily processed by all thermoplastic techniques are some of the advantages of HDPE. Some of the disadvantages include high thermal expansion, flammable, poor resistance to weathering, stress cracking, difficulty to bond, and poor temperature capability. Table 2.5 below shows a summary of some differences in the properties of LDPE and HDPE resins.

**Table 2.5. Temperature Permeability Reference Chart, 2016**

| Resin | Max Used Temp ($^{o}$C) | HDT (C) | Flexibility | Permeability (cc-mil/100in$^2$-24hr.-Bar) | | | Water Vapour Transmission Rate (g-mm/m$^2$-24hr.-Bar at 38C, 90% RH) |
|---|---|---|---|---|---|---|---|
| | | | | $N_2$ | $O_2$ | $CO_2$ | |
| LDPE | 80 | 45 | Excellent | 180 | 500 | 2700 | 15.5-23.3 |
| HDPE | 120 | 65 | Rigid | 42 | 185 | 580 | 4.6-6.2 |

(Source: www.calpaclab.com/reference-chart-temperature/)

## 2.6. SOURCES OF DRINKING WATER AND TREATMENT SYSTEM IN GHANA

In Ghana, the main source of water for drinking is the municipal water (pipe-borne water). Hand-dug borehole water is now on the increase in some parts of the country.

### 2.6.1 Pre-Packaging Treatment (Water Treatment Process) of sachet water

Source (pipe-borne/borehole water)
↓
Water Storage Tanks
↓
Multi-grade Sand Filter
↓
Activated Carbon Filter
↓
Ion Exchanger (if source is a borehole with lots of metal ions concentration)
↓
Filter Cartridge
↓
Reverse Osmosis Membrane
↓
Storage Tank
↓
Filter Cartridges (Carbon filters, polishing filters)
↓
UV Sterilizer
↓
Automated Filling/Packaging Machine

**Figure 2.10. A Typical Water Treatment Process Prior to Packaging into Sachets**

**Figure 2.11. A water treatment plant for sachet water**

## 2.7. CONTAMINATION OF WATER DURING AND/OR AFTER TREATMENT

Presence of particles in packaged water, mostly come from the source of the raw water; however, some of the particles could stay in the water after treatment due to poor or insufficient treatment procedures and handling. Product contamination could result from the processed water, or subtly from the production activities like cleaning activities, contact surfaces of materials to be used for packaging, and/or cross-contamination from wet surfaces of the production area, floors, the processing equipment [Baird and Petrie, (1981) as cited in Jeje and Oladepo, (2012)]. Mendie (2001) (as cited in Jeje and Oladepo, 2012) asserts that the environment where the water is treated and packaged could contribute to extrinsic contaminants of the water. Production and filling environment, the processing equipment and operatives comprise the macro-environment, while the primary water packaging defines the micro-environment. The system of controls of physical, chemical, and microbiological factors influencing the manufacture of packaged waters, and the level of each can therefore result in the presence of pollutants.

According to Jeje and Oladepo, (2012), the semi-permeable membrane through which water is treated via reverse osmosis can result in a germ-free and pathogen-free product since the mechanism of action only permits substances with molecular weight of 250 Daltons or less to pass through. However, cross-contamination can arise after the process due to the entry of microorganisms into the storage vessel from the membrane downstream of the distribution system.

Carbon cartridge filters are efficient in eliminating odour, chlorine, lower molecular weight hydrocarbons and oxygen; however, they are not so efficient in eliminating high molecular weight organic materials (www.uspurwater.com). Carbon filters are usually included in water treatment process to reduce permanent pollution of the deionizing bed and organic molecules trapped onto, and held inside the activated carbon particles that sustain growth of

microorganisms. Usually, the maximum numbers of fouling biota are present at the underneath of the bed as residual chlorine is eliminated at the upper section. Common microorganisms recovered from carbon filters include: *Pseudomonas* spp., *Arthrobacter,* Coliforms, *Micrococcus, Alcaligenes and Corynebacter* [Favero *et al*. (1971) as cited in Jeje and Oladepo, (2012)].

In the treatment of raw water high in calcium and magnesium levels, water softener is essential on the treatment line. Generally, they are more likely to be microbially contaminated as compared to anion or cation exchange resins since they are recharged with acid and strong alkali that have strong bactericidal effects. Periodic bactericidal effects are not observed for the softeners using sodium chloride regeneration solutions. Also, brine tanks in which the salt solutions are prepared for the regeneration or recharging of the water softener tend to be polluted with halophiles and other salt-tolerant microorganisms if proper precautionary measures are not put in place [Baird and Shooter, (1978) as cited in Jeje and Oladepo, (2012)]. Ion-exchange resins or water softeners once maintained properly do not pose any significant problem with contamination with microorganisms. Nonetheless, after an extended period of not in use, microbial contamination may occur due to the presence of some types of organic material in the input water. Microorganisms often connected with deionizers include *Pseudomonas, Acinetobacter*, and *Alcaligenes* spp. [Baird and Shooter, (1978) as cited in Jeje and Oladepo, (2012)]. The installation of UV sterilizers on the water treatment lines may be used to control the microbial load if the flow rate of water is adjusted to that of the sterilizer and the burning hours of the sterilizer has not expired.

Water storage tanks if not cleaned and sanitized well also contribute to microbial growth and contamination with particles. Chemical and physical contaminants can rather be easily minimised at the pre-production stages, unlike microbial contaminants which require proper cleaning and sanitizing programmes to be adhered to strictly (Jeje and Oladepo, 2012). Table

2.6 shows how some microorganisms can be eliminated from drinking water.

Generally, the compliance to the current codes of Good Manufacturing Practices (cGMP) guidelines helps to lower microbial loopholes to the barest minimum in the packaged water. To achieve microbial safety of food by adherence to the current codes of Good Manufacturing Practices, the Food and Drugs Authority in Ghana, licenses all Food Manufacturing Facilities including the sachet water manufacturing facilities. The manufacturing facilities must obtain their license from the Food and Drugs Authority before the Authority registers the sachet water. Table 2.6 indicates how pathogens and indicators are eliminated from drinking water.

**Table 2.6. How Pathogens and Indicators are eliminated from Drinking Water.**

| **Pathogen or Indicator** | **Treatment methods proven to be effective for removal or inactivation** |
|---|---|
| *Cryptosporidium* | Sterilization by ozone or ultraviolet sterilizer and/or filtration. |
| *Giardia lamblia* | Filtration and/or use of chlorine. |
| *Legionella* | Filtration and/or use of chlorine. |
| Viruses (enteric) | Filtration and/or use of chlorine. |
| Total coliforms, faecal Coliform, *E. coli* (NOT including *E. coli O157:H7)* and faecal indicators (Coliphage or Enterococci*)* | Sterilization by chlorine of correct concentration, ozone or use of ultra-violet sterilizer. These help to inactivate or kill *E. coli.* |

(Source: EPA, 2013)

## 2.8. MICROORGANISMS ISOLATED FROM PACKAGED WATER (SACHET)

Many works have been carried out on packaged water (Sachet Water) in Ghana (Obiri-Danso *et al*., (2003); Kwakye-Nuako *et al*., (2007); Osei *et al*., (2013). In Kumasi, Ghana, Obiri-Danso *et al*. (2003) examined 88 sachet water (factory-filled), 40 hand-filled-and-tied polythene-bagged and 8 bottled water for the presence of total viable counts (TVCs), heterotrophic bacteria, and faecal contamination indicators (total coliforms, enterococci, and faecal coliforms). Their investigation revealed heterotrophic bacteria in all 3 water types with TVCs per ml ranging from 2-6.33 x $10^5$ for sachet water (factory-bagged), 2.33 x $10^3$-7.33 x

$10^{12}$ for hand-filled-and-tied bagged water and 1-460 for bottled water. No microbial indicators of faecal contamination were identified in the bottled water. However, 4.5% of the sachets (factory-bagged) had 2.3% faecal coliforms and total coliforms, while 42.5% of the hand-filled-and-tied bags contained total coliforms, 5% enterococci and 22.5% faecal coliforms.

Work done in Accra, Ghana by Kwakye-Nuako *et al*. (2007), identified 77% of the samples containing parasitic pathogenic microbes at the infective stage. Common pathogens classified included, *Microsporidia* spp 14/27 (51.2%), *Sarcocystis* spp. 18/27 (66.7%), *Cryptosporidium parvum* 17/27 (63.0%), Rotifers 5/27 (18.5%), *Cyclospora cayetenensis 16/27* (59.3%) and Charcoal Leyden crystals 12/27 (44.4%). About 93% of samples had unidentified impurities with 29.6% of the samples containing at least one type of parasite, 14.8% containing at least 2 types of parasites, 25.9% containing at least three types of parasites, while 29.6% contained 4 types of parasites.

Osei *et al*. (2013), worked on 60 sachet water and 10 of bottled water samples purchased randomly from different locations in the Accra Metropolis. Results on the bacteriological analyses showed that, 8 out of the 60 samples (13.3%) of sachet water had heterotrophic plate count (HPC) levels within the recommended limits of 500 cfu/ml (WHO, 2004). The remaining 52 (86.7%) had HPC levels well above the recommended limit of 500 cfu/mL (WHO, 2004). For the samples of bottled water, 9 out of 10 (90%) were within the HPC recommended limit. Only 1 (10%) sample had levels above the recommended limits.

Olaoye and Onilude (2009) evaluated the microbial quality of 92 packaged sachet water in Western Nigeria and its impact on public health. Their results reported total bacteria count ranging between 2.86 and 3.45 log cfu/ml. The highest coliform count was 1.62 log cfu/ml whereas faecal coliform (*E. coli)* was detected in 2.2% of total samples. Amongst the identified bacteria from the water samples included *E. coli, Pseudomonas aeruginosa, Alcaligenes faecalis, Enterobacter aerogenes, Proteus vulgaris, Klebsiella* spp., *Bacillus cereus,*

*Staphylococcus aureus*, *Streptococcus lactis*, *Aeromonas* spp., and *Micrococcus luteum*. They concluded that some of the packaged water (sachet) were of poor quality and appropriate regulatory measures should be taken to safe guard public health and safety.

Akinde *et al.* (2011) investigated the quality of 10 NAFDAC-certified sachet water brands produced within 24 hours and during storage at room temperature. They reported that total aerobic heterotrophic bacterial count gradually increased in all brands to unacceptable limits within four weeks of storage and gradually decreased to undetectable levels by the time the experiment ended. Total and faecal coliforms were found in 40% of analyzed water samples within the initial 3 weeks, but were not identified thereafter. *E. coli* was isolated in one brand at the onset, while faecal coliform and streptococci were not identified throughout storage. About sixty percent (60%) of the analyzed brands met the WHO standard limit for drinking water when stored at ambient temperature within a 4-week period. Beyond 4-weeks storage however, reduced aesthetic quality and increased growth of bacteria to harmful levels in the sachet water were observed.

Ezeugwunne *et al*. (2009), worked on 90 sachet water samples during an investigation concerning the portability of sachet water. The percent isolation of microbes at the end of the study in the water samples revealed that *E. coli* had the maximum occurrence rate (36.1%), followed by *S. aureus* (25.0%), *S. faecalis* (19.4%) and *K. pneumoniae* (11.4%). Viable counts for some of the water samples were in the range of $1.0\times10^4$-$1.6\times10^4$ cfu/ml, $2.0\times10^3$-$2.6\times10^3$ cfu/ml and $2.0\times10^3$-$2.6\times10^3$ cfu/ml whilst other samples were satisfactory. Adekunle *et al.* (2004), identified bacterial growth in sachet water during a study, which revealed the presence of *E. coli, Kiebsiella* spp*, Pseudomona aeruginosa and Streptococcus faecalis.* Akorli (2012), showed that brands of bottled water were not contaminated with total coliforms, faecal coliforms or *Escherichia coli* whilst 55.6% of sachet water brands were reported to be contaminated with total coliforms, faecal coliforms and *Escherichia coli*.

### 2.8.1. Growth of Microorganisms in Water

Normally, microorganisms grow in water and on surfaces in contact with water as biofilms. Growth of microorganism in the water after the water has been treated is known as "regrowth." The growth is usually revealed in higher Heterotrophic Plate Count (HPC) values measured in water samples (Bartram *et al*., 2003).

During a presentation by Alburelkan (2015), she stated that microbial growth is an autocatalytic process where growth of microorganisms does not occur without the presence of at least one viable cell. Todar (2012), asserts that at the exponential phase of growth, all the cells of the microorganisms are dividing regularly and growing geometrically; however, the rate of cell division is dependent on the composition of the growth medium and the conditions of incubation.

### 2.8.2. Parameters of Concern in Drinking Water Quality

#### 2.8.2.1. Total bacteria count

TBC (Total Bacterial Count) also identified as heterotrophic plate count (HPC), aerobic plate count, (APC), heterotrophic colony count (HCC), standard plate count (SPC) or total plate count (TPC), is the total bacterial counts in a particular sample. These tests identify all viable microorganisms that can grow on plate count agar aerobically at suitable incubation condition (usually 37 $^{o}$C, 48 hrs). Total Bacterial Count analyses reveal the usual hygiene state of a sample (http://www.envirolabs.com.hk, 2011).

Heterotrophs are collection of microorganisms (bacteria, moulds and yeast) which make use of sources of organic carbon to grow and can be found in all types of water. They form majority of bacteria identified in drinking water systems (www.moldbacteriaconsulting.com).

Heterotrophic Plate Count does not have health effects; it is an analytical technique that measures the diversity of bacteria that are common in water. The lesser the bacteria count in

drinking water, the better the water system has been maintained. HPC tests were used as signs that the processes (particularly the sand filtration process) are functioning properly and thereby indirectly indicating the safety of the water. HPC measurements nonetheless continue to feature in the water guidelines or regulations of water in various countries. HPC measurements are usually used as indicator for determining the efficiency of treatment processes the water undergoes (Bartram *et al.,* 2003).

Total Plate Count (TPC) measures the biological activity of microorganism in a sample. It is a count of moulds and yeasts and all heterotrophic bacteria, which will grow in aerobic (oxygen-loving) or microaerophilic or mesophilic (moderate-temperature-loving) conditions. It has been stated that the TPC test is done to be able to assess the effectiveness of a treatment process of drinking water, to determine the safety of a water supply or product, and to measure a product (such as food) deterioration or 'shelf-life (www.mblabs.com).

#### 2.8.2.2. Coliforms

Coliforms are found in faeces and present naturally in the environment. *E. coli* and faecal coliforms only come from animal and human faeces. Total coliform test is not a health hazard in itself but used to reveal the presence of other possibly harmful bacteria (www.doh.wa.gov).

#### 2.8.2.3. pH

pH influences growth and rate of growth of microorganisms. Microbes have optimum pH level at which they grow rapidly and range of pH with which they will not grow. Some microorganisms grow at neutral pH or slightly alkaline (bacteria) and some grow at low pH (yeasts and moulds and some bacteria). Some bacteria can grow at very high pH of 11 and some as low as 4 (Vieira, 1999).

Carbon dioxide–bicarbonate–carbonate equilibrium system has an effect on the pH of most natural waters. Decrease in carbon dioxide concentration raises pH and an increase will cause it to reduce (WHO, 2003). The equilibria of pH is also affected by temperature. In pure water, when temperature is raised by 25 °C there is a reduction in pH of about 0.45 (WHO 2003).

## 2.9. REGULATION OF THE USE OF PLASTICS FOR WATER PACKAGING

The Food and Drug Administration, in the United States, regulates the safety of food contact materials used in packaging. These include plastics used in contact with food. For decades many plastics such as polyethylene and polystyrene have been used in food packaging. Before all food-contact packaging materials can be displayed on the market for sale, they are supposed to be found safe for use in their specified applications after going through the rigorous and strict approval process of FDA (Russell, 2015).

According to the World Packaging Organisation (2009), the packaging materials selected to package food must conform to current regulation in place. Regulations such as the EU Regulation 1935/2004 (Materials and Articles in Contact with Food Regulations) and corresponding requirements of FDA in the USA. Plastics as packaging materials especially with regards to specific requirements of the materials used should be given attention. The FDA CFR 21 and 2002/72/EU and its 5 amendments, indicate the precise quantities and kinds of additives that can be used in the production of the plastics and each additive having been examined and accepted for food use. The dealer of packaging and packaging materials must produce suitable compliance documents, such as Safety Data Sheets and Food Contact Statements. This is to certify that any migrations from the essential additives used in the production of plastic are maintained within tightly regulated limits. The migration requirement of the finished products, thus the plastic must be met (World Packaging Organisation, 2009).

The World Packaging Organisation (2009), also indicates that various quality and hygiene

systems, for example, ISO 22000, the BRC/IOP Global Standard for Packaging and Packaging Materials and ISO EN 15593, have been established to help guarantee the safety and hygienic manufacturing of packaging that comes into contact with food. Packaging manufacturers in order to improve their production facilities and ensure the greatest quality in their products are adopting these Standards worldwide

In Ghana, however, the Food and Drugs Authority only registers products and not the packaging. This is because the country does not have a Packaging Policy in place. A Packaging Policy Committee has now been formed to help develop Packaging Policy for regulatory and monitoring purposes.

# CHAPTER THREE

## 3.0. MATERIALS AND METHODS

### 3.1. SAMPLING

Ninety (90) pieces (500 ml) of stock sachet water were packaged in Low Density Polyethylene (LDPE) and another ninety (90) pieces (500 ml) of stock sachet water in High Density Polyethylene (HDPE) from the same water producing and packaging company within the same time period were used for this work. The samples were transported within twenty-four (24) hours in ice-chest containers to the laboratory for microbiological analysis (Total Plate Count and Total Coliform Count), pH and temperature.

The microbiological, pH and temperature determinations of the sachet water with the different packaging materials were carried out immediately and subsequently on weekly basis for a six (6) week storage period. Samples of three (3) were drawn from the stock samples and analyzed during each period of analysis.

The 90 pieces of the water samples in each of the packaging types (HDPE and LDPE) were stored under three conditions of room temperature, refrigeration temperature and the other samples exposed to the sun over a period of 6 weeks. Thus, 30 pieces each of the sachet water in HDPE and LDPE were refrigerated; another 30 pieces each were exposed to sunlight whilst the remaining 30 pieces each were stored at room temperature. The samples exposed to the sun were brought out daily into the open and returned into the storage room for the period of six (6) weeks storage time. This is to depict how sachet water is vended.

Samples in HDPE and LDPE under refrigeration were coded HDF and LDF respectively. Samples in HDPE and LDPE stored at room temperature were coded HDR and LDR respectively. Samples in HDPE and LDPE exposed to sunlight were coded HDS and LDS respectively. Three samples from the three storage conditions were taken and analyzed in triplicates on weekly basis for six weeks.

## 3.2. PHYSICOCHEMICAL PARAMETERS

### 3.2.1. Determination of pH and Temperature

Each water sample was cooled to ambient temperature. The pH/conductivity-meter (JENWAY 3540 pH and Conductivity Meter model) was calibrated using pH buffers 4.01 and 7.01. The thermometer was also calibrated before taking readings. The pH and the temperature probes were rinsed with distilled water and wiped off before measurement. The pH and temperature probes were inserted into the sample water, agitated and the readings were allowed to be stable before being recorded.

## 3.3. MICROBIOLOGICAL ANALYSES

### 3.3.1. Determination of Total Coliform Count (TCC)

The detection and enumeration of Total Coliform in the water was performed using the Horizontal method for the detection and enumeration of coliforms – Most Probable Number Technique (ISO 4831: 2006).

Briefly, ten milliliters (10 ml) of the water was transferred into 90 ml of sterilized buffered peptone water and homogenized in a water bath at 45 °C. All the test tubes used contained an inverted Durham tube.

A set of five (5) test tubes, each containing 10 ml double-strength of sterilized Lauryl Tryptose/Sulphate Broth were inoculated using a sterile pipette with 10 ml of the initial solution to form 10 dilution. Another set of five (5) test tubes, each containing 9 ml single-strength of sterilized Lauryl Tryptose/Sulphate Broth was inoculated using a sterile pipette with 1 ml of the initial solution to form $10^{-1}$ dilution. A separate set of five (5) test tubes, each containing 9 ml single-strength of sterilized Lauryl Tryptose/Sulphate Broth was again inoculated using a sterile pipette with 0.1ml of the initial solution to form $10^{-2}$ dilution. The set of tubes were incubated at 37 °C for 24 ± 2 h. The tubes were examined after the incubation period for gas

formation or opacity/cloudiness in the Durham tubes. All the tubes were incubated again for another 24 h ± 2 h, when neither gas formation nor opacity was observed.

The results were confirmed by inoculating 0.5ml each from the positive Lauryl Tryptose/Sulphate Broth into 5 ml MacConkey bottles containing 4.5 ml each of sterilized Brilliant Green Lactose Bile Broth and incubated at 37 $^{o}$C for 24 h ± 2 h. The tubes were examined after the incubation period for gas formation or opacity/cloudiness that prevented the observation of gas production in the Durham tubes. Incubation was repeated for another 24 h ± 2 h when neither gas formation nor opacity was observed

For the interpretation and expression of results, the standardized 5-tube Most Probable Number (MPN) table (Table 3.1) was used for each dilution in which gas formation was observed. This indicated the presence or absence of coliforms in a test portion of sample.

**Table 3.1. Most Probable Number values per gram of sample and 95% confidence limits**

| No. positive results for inoculum volume (ml or g) | | | MPN/ml or / g | $Log_{10}$ MPN | SD $log_{10}$ MPN | 95% Confidence Limits | | Rarity Index | Category |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.1 | 0.01 | | | | Lower | Upper | | |
| 0 | 0 | 0 | 0 | N/ A | N/A | 0 | 0.66 | 1 | 1 |
| 0 | 1 | 0 | 0.18 | -0.74 | 0.43 | 0.02 | 1.34 | 0.09 | 1 |
| 1 | 0 | 0 | 0.20 | -0.70 | 0.44 | 0.03 | 1.47 | 1.00 | 1 |
| 1 | 0 | 1 | 0.40 | -0.40 | 0.31 | 0.10 | 1.65 | 0.02 | 2 |
| 1 | 1 | 0 | 0.40 | -0.39 | 0.31 | 0.10 | 1.66 | 0.21 | 1 |
| 1 | 2 | 0 | 0.61 | -0.21 | 0.25 | 0.19 | 1.96 | 0.02 | 2 |
| 2 | 0 | 0 | 0.45 | -0.35 | 0.31 | 0.11 | 1.86 | 1.00 | 1 |
| 2 | 0 | 1 | 0.68 | -0.17 | 0.25 | 0.21 | 2.18 | 0.03 | 2 |
| 2 | 1 | 0 | 0.68 | -0.16 | 0.25 | 0.21 | 2.2 | 0.35 | 1 |
| 2 | 1 | 1 | 0.92 | -0.04 | 0.22 | 0.33 | 2.55 | 0.02 | 2 |
| 2 | 2 | 0 | 0.93 | -0.03 | 0.22 | 0.34 | 2.58 | 0.06 | 1 |
| 3 | 0 | 0 | 0.78 | -0.11 | 0.26 | 0.24 | 2.54 | 1.00 | 1 |
| 3 | 0 | 1 | 1.1 | 0.03 | 0.23 | 0.38 | 2.97 | 0.05 | 1 |
| 3 | 1 | 0 | 1.1 | 0.03 | 0.23 | 0.38 | 3.02 | 0.57 | 1 |
| 3 | 1 | 1 | 1.4 | 0.14 | 0.20 | 0.54 | 3.48 | 0.03 | 2 |
| 3 | 2 | 0 | 1.4 | 0.14 | 0.20 | 0.54 | 3.53 | 0.15 | 1 |
| 3 | 2 | 1 | 1.7 | 0.23 | 0.19 | 0.72 | 4.02 | 0.13 | 2 |
| 3 | 3 | 0 | 1.7 | 0.24 | 0.19 | 0.73 | 4.09 | 0.03 | 2 |
| 4 | 0 | 0 | 1.3 | 0.11 | 0.23 | 0.44 | 3.72 | 1.00 | 1 |
| 4 | 0 | 1 | 1.7 | 0.22 | 0.21 | 0.63 | 4.4 | 0.08 | 1 |
| 4 | 1 | 0 | 1.7 | 0.23 | 0.21 | 0.63 | 4.5 | 0.92 | 1 |
| 4 | 1 | 1 | 2.1 | 0.33 | 0.20 | 0.85 | 5.28 | 0.07 | 1 |
| 4 | 2 | 0 | 2.2 | 0.33 | 0.20 | 0.86 | 5.41 | 0.31 | 1 |
| 4 | 2 | 1 | 2.6 | 0.42 | 0.19 | 1.1 | 6.31 | 0.03 | 2 |
| 4 | 3 | 0 | 2.7 | 0.43 | 0.19 | 1.1 | 6.5 | 0.07 | 1 |
| 4 | 4 | 0 | 3.4 | 0.53 | 0.18 | 1.5 | 7.8 | 0.01 | 2 |
| 5 | 0 | 0 | 2.3 | 0.36 | 0.24 | 0.76 | 7.0 | 0.77 | 1 |
| 5 | 0 | 1 | 3.1 | 0.50 | 0.24 | 1.1 | 9.4 | 0.09 | 1 |
| 5 | 1 | 0 | 3.3 | 0.52 | 0.24 | 1.1 | 10 | 1.00 | 1 |
| 5 | 1 | 1 | 4.6 | 0.66 | 0.25 | 1.5 | 14 | 0.20 | 1 |
| 5 | 1 | 2 | 6.3 | 0.80 | 0.24 | 2.1 | 19 | 0.02 | 2 |
| 5 | 2 | 0 | 4.9 | 0.69 | 0.26 | 1.5 | 16 | 1.00 | 1 |

(When five test portions of 1g, five of 0.1g and five of 0.01g are used)

Source: (ISO 4831, 2006)

### 3.3.2. Determination of Total Plate Count

The detection and enumeration of Total Plate Count was perfumed by the Horizontal method for the enumeration of microorganisms – colony count at 30 $^{o}$C by the Pour Plate Technique (ISO 4833-1: 2013). 10 ml water sample was weighed into 90 ml of sterilized buffered peptone water and homogenized in a water bath at 42-45 °C. By means of a sterile pipette, 1 ml of initial solution ($10^{-1}$ dilution) was transferred into 9 ml of sterilized buffered peptone water and homogenized to form $10^{-2}$ dilution. Using a sterile pipette 1 ml each of the $10^{-2}$ dilution was transferred into two sterile Petri dishes, appropriately labeled. About 20-25 ml of molten plate count agar (containing 1% 2,3,5-Triphenyltetrazolium chloride solution, TTC - 1ml to each 100 ml plate count agar) at 42-45 °C was poured into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes was within 45 min.

The sample dilution and agar medium were mixed carefully but immediately and thoroughly by alternate rotation followed by back and forth movement of the dish on the flat smooth working surface. The mixture was allowed to solidify by leaving on the cool working surface for about 5 minutes. The prepared dishes were inverted and were placed in the incubator at 30 °C ± 1 °C for 72 h ± 3 h. The dishes were not stacked more than six high. The stacks of dishes were separated from one another and from the walls and top of the incubator. A control test was done to check the sterility of the Plate Count Agar by pouring the agar media into a Petri dish and was also incubated at 30 °C ± 1 °C for 72 h ± 3 h. The 1% 2,3,5- Triphenyltetrazolium chloride solution, TTC is a staining agent that differentiates between active and inactive tissue (living and nonliving) since it is enzymatically reduced to deep red in living tissue.

The colonies on the plates were counted after the specified incubation period using the colony counter and recorded. Each dish's count was multiplied by its dilution factor, thus 100 because

of ($10^{-2}$ dilution). The average of the two retained dishes was estimated for each sample and recorded in unit of cfu/ml as the TPC result.

### 3.4. STATISTICAL ANALYSIS

A 2-way analysis of variance was performed to analyze effects of packaging type and storage time on TPC, temp and pH of packaged water. Pearson correlation coefficients between TPC and pH, and TPC and temperature were also computed for each packaging type and storage condition. All data analysis was conducted using SPSS at a 95% confidence level.

# CHAPTER FOUR

## 4.0. RESULTS

### 4.1. pH OF THE STORED SACHET WATER

Results of the effect of packaging type on the pH of the stored sachet water are presented in Figure 4.1. The pH of the sample water in the fresh state was at an average of 7.13 ± 0.06. At week one (1) under the different storage conditions and the packaging materials, almost all average pH values increased between a range of 7.18 ± 0.11 for HDF to 7.44 ± 0.02 for LDS; except for LDF, which reduced to 7.05 ± 0.27. At the end of the second week of storage, the pHs reduced within a range of 7.02 ± 0.03 for LDF to 7.08 ± 0.02 for HDS and LDR. Week three recorded an increase in pH values for all the samples in the different packaging materials under the different storage conditions. It ranged from 7.30 ± 0.14 for LDS to 7.46 ± 0.12 for LDR. At 95% confidence level, there was no significant difference ($p > 0.05$) in the pH changes with the initial pH of the samples over the three weeks storage period except for LDF in the first week. The pH reduced at the end of the fourth week for all samples from 6.35 ± 0.06 for LDF to 7.02 ± 0.02 for HDR. LDR and LDF had the highest reduction; from 7.46 ± 0.12 to 6.45 ± 0.10 and 7.37 ± 0.07 to 6.35 ± 0.06, respectively. At the end of the fourth week, changes in pH levels were significant for LDF, HDS and HDF at $P > 0.05$. At week five (5), there was an increase in pH values for all samples within the range of 7.16 ± 0.03 for HDF to 7.34 ± 0.07 for LDF. At end of the sixth ($6^{th}$) week, all three of the samples in LDPE recorded marginal reductions in pH values compared to the fifth week results, ranging from 7.27 ± 0.06 for LDR to 7.32 ± 0.04 for LDF. Samples in HDPE recorded marginal increases in pH values compared to the fifth week results with pH values ranging from 7.19 ± 0.17 for HDF to 7.33 ± 0.08 for HDS.

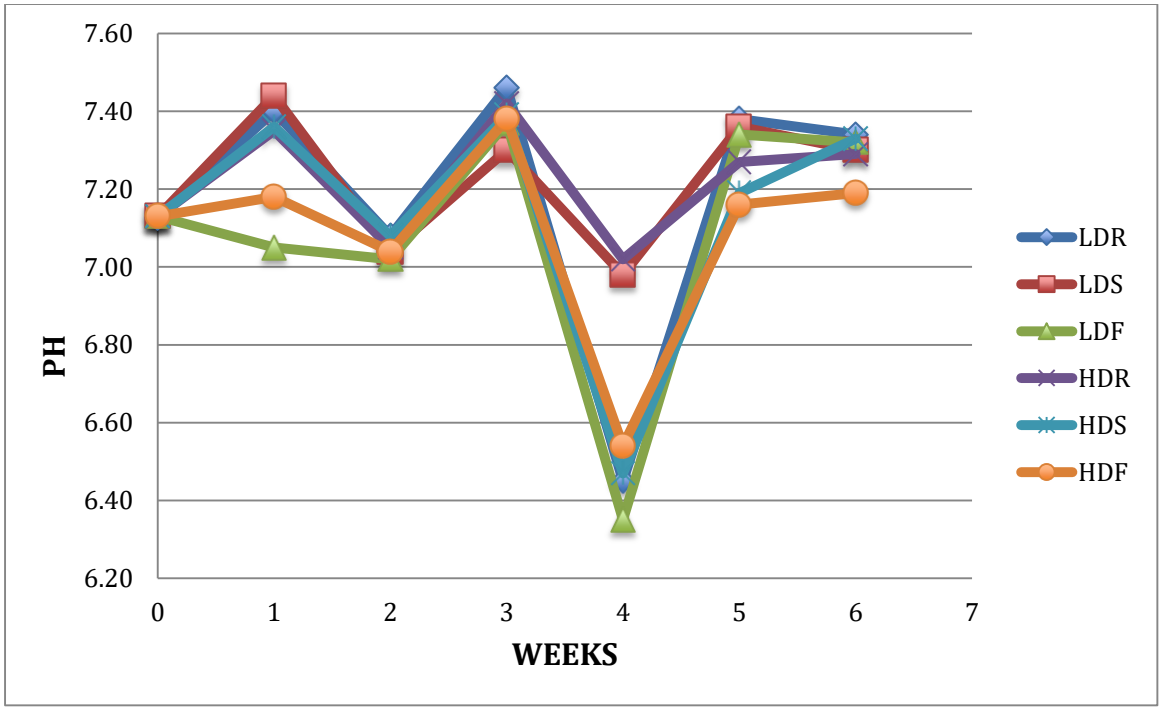


**Figure 4.1. Effect of packaging (Polyethylene) type on the pH of the stored sachet water**

**KEY:**
LDR and HDR water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under room temperature storage
LDS and HDS water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under sunlight exposure
LDF and HDF water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under refrigeration storage

## 4.2. TEMPERATURE OF THE STORED SACHET WATER

Results of the effect of packaging type on the temperature of the stored sachet water are presented in Figure 4.2. In this study, the freshly packaged water samples had an average temperature of 24.50 ± 0.0 °C. At the end of week one, there was an increase in temperature for all samples ranging from 27.50 ± 0.26 °C for LDF, to 30.17 ± 0.25 °C for LDS. The temperature for the LDF was found to be significantly different from the temperature of the initial fresh water (at 95% confidence interval). At the end of week two, three samples (HDF, LDF and LDR) showed rise in temperature ranging from 29.13 ± 0.21 °C for LDF to 29.70 ± 0.34 °C for LDR. The other three (HDS, LDS and HDR) recorded decreases in temperature ranging from 28.9 ± 0.12 °C for HDS to 29.2 ± 0.31 °C for HDR. These variations in

temperature were, however, not significantly different ($p > 0.05$) from that of the fresh sample. The temperature for all the samples decreased at the end of the third week with LDS having the least decrease from 29.50 ± 0.20 °C to 29.03 ± 0.15 °C. LDF recorded the most decrease from 29.13 ± 0.21 °C to 24.77 ± 0.31 °C, which was significantly different at 95% confidence interval. At week four, LDR, HDS, LDF showed an increased in temperature while LDS, HDR and HDF decreased in temperature. The increase in temperatures for LDS (29.03 ± 0.15 °C) and HDF (28.43 ± 0.06 °C) were significantly different from the fresh sample at 95% confidence interval.

At the end of week five, the temperature for all the samples reduced in a range of 24.60 ± 0.10 °C for LDF to 25.07 ± 0.06 °C for LDR. The reductions in temperature were significantly different at 95% confidence interval for LDS, LDF, HDS and HDF. Week six results showed a slight decrease in temperatures for LDR at 23.47 ± 0.06 °C and HDF at 24.37 ± 1.67 °C and a slight increase for LDS at 24.83 ± 2.66 °C and LDF at 25.60 ± 1.85 °C. HDS recorded 27.17 ± 0.29 °C as the highest increase in temperature with no significant difference ($p > 0.05$) to the initial temperature of sample water.

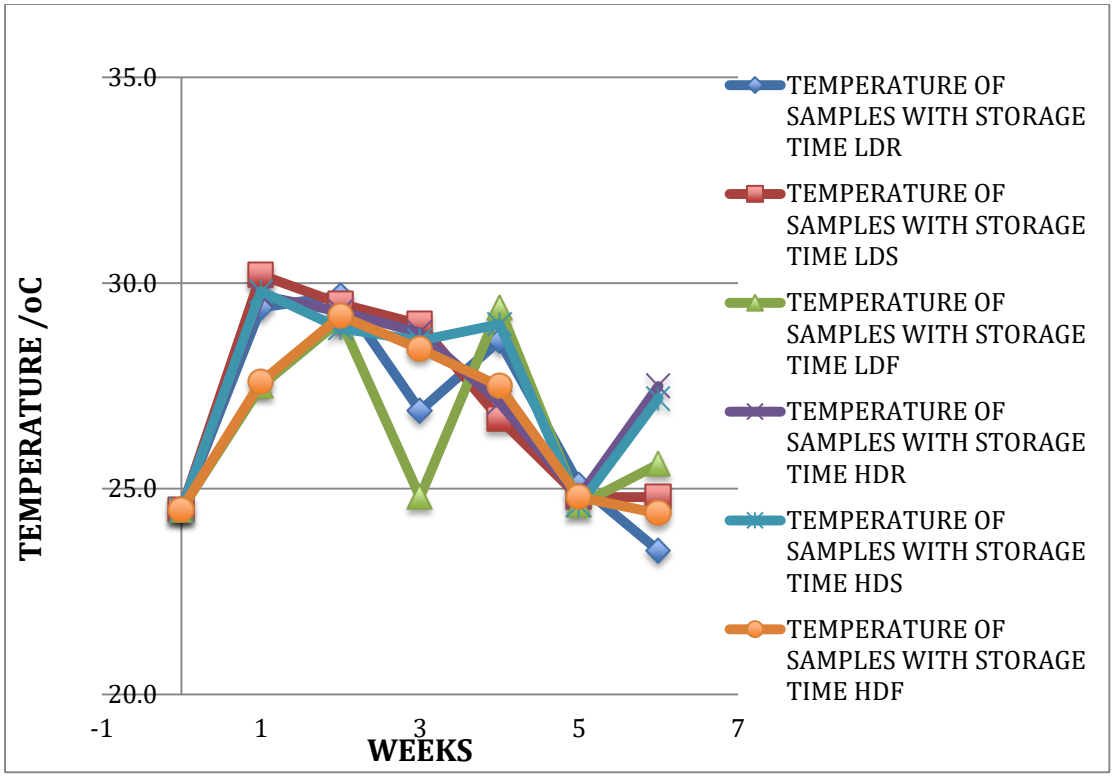


**Figure 4.2. Packaging (Polyethylene) type on the temperature of the stored sachet water**

## 4.3. MICROBIAL COUNTS OF THE TEST SAMPLES

Results of the effect of packaging type on the total plate count of the stored sachet water are presented in Figure 4.3. The average total plate count (TPC) for the freshly packaged water samples was log 2.22 ± 0.07 cfu/ml. In the first and second weeks LDR had the highest TPC of log 2.89 ± 0.04 cfu/ml and log 2.88 ± 0.03 cfu/ml respectively, and these were significantly different from the initial fresh state at $p>0.05$. HDS recorded the lowest TPC of log 2.26 ± 0.07 cfu/ml within these weeks but was not significantly different ($p < 0.05$) from the initial value of log 2.22 ± 0.07 cfu/ml.

TPC levels increased in LDF increased from log 2.76 ± 0.06 cfu/ml to log 2.87 ± 0.05 cfu/ml from week one to week three respectively. After the third week, the TPC reduced to log 2.83 ± 0.04 cfu/ml and log 2.82 ± 0.05 cfu/ml in weeks four and five respectively. The increase in TPC level in the first week was significantly different from the fresh state ($p>0.05$). Changes in TPC level in the subsequent weeks to the fifth week were not significantly different from

week one ($p>0.05$). In the sixth week, however, the TPC reduced further to log 2.54 ± 0.06 cfu/ml and was significantly different ($p<0.05$) from all the others.

TPC level at end of the first ($1^{st}$) week in LDS increased to log 2.67 ± 0.06 cfu/ml, slightly decreased to log 2.63 ± 0.03 cfu/ml at the end of the $2^{nd}$ week and increased in the subsequent weeks. Week three (3) had a slight increase in TPC level of log 2.65 ± 0.05 cfu/ml after which the subsequent three weeks recorded a reduction in TPC levels. At week six, the TPC level was log 1.80 ± 0.17 cfu/ml. The TPC levels were not significantly different at $p>0.05$.

There was an increase in TPC level in HDF from weeks one and two which were significantly different ($p<0.05$) from the initial TPC. The TPC level reduced at week three and increased from weeks four to five with no significant difference ($p>0.05$) from the first week. On the sixth week, the TPC level reduced to log 2.12 ± 0.10 cfu/ml which was significantly different ($p<0.05$) from the previous five weeks but not significantly different from the initial fresh state.

HDR had its TPC level increasing from the initial level to the sixth week. In the first week, the TPC averaged at log 2.44 ± 0.12 cfu/ml and was significantly different ($p<0.05$) from the subsequent weeks and the initial fresh state. The TPC at the sixth week was log 2.98 ± 0.03 cfu/ml. There was no significant difference ($p>0.05$) for TPC levels for the second and third weeks and for the fourth, fifth and sixth weeks. The TPC levels during the storage periods were significantly different ($p<0.05$) from the TPC levels in the initial fresh sample.

The TPC level for HDS increased to log 2.26 ± 0.07 cfu/ml at the end of the first and second weeks, after which the level decreased gradually to the fifth week with no significant difference ($p>0.05$) from the initial TPC values. At the end of the sixth week, the TPC reduced to log 0.57 ± 0.98 cfu/ml. The reduction was significantly different from the previous levels.

HDR showed increase in TPC levels from the first ($1^{st}$) week to the fifth ($5^{th}$) week within a range of log 2.64 ± 0.14 cfu/ml to 2.72 ± 0.08 cfu/ml, respectively. These increases were significantly different from the fresh state at $p>0.05$. At the end of week six (6), the TPC level

reduced to log 2.12 ± 0.10 cfu/ml but was not significantly different from the initial TPC level (fresh state).

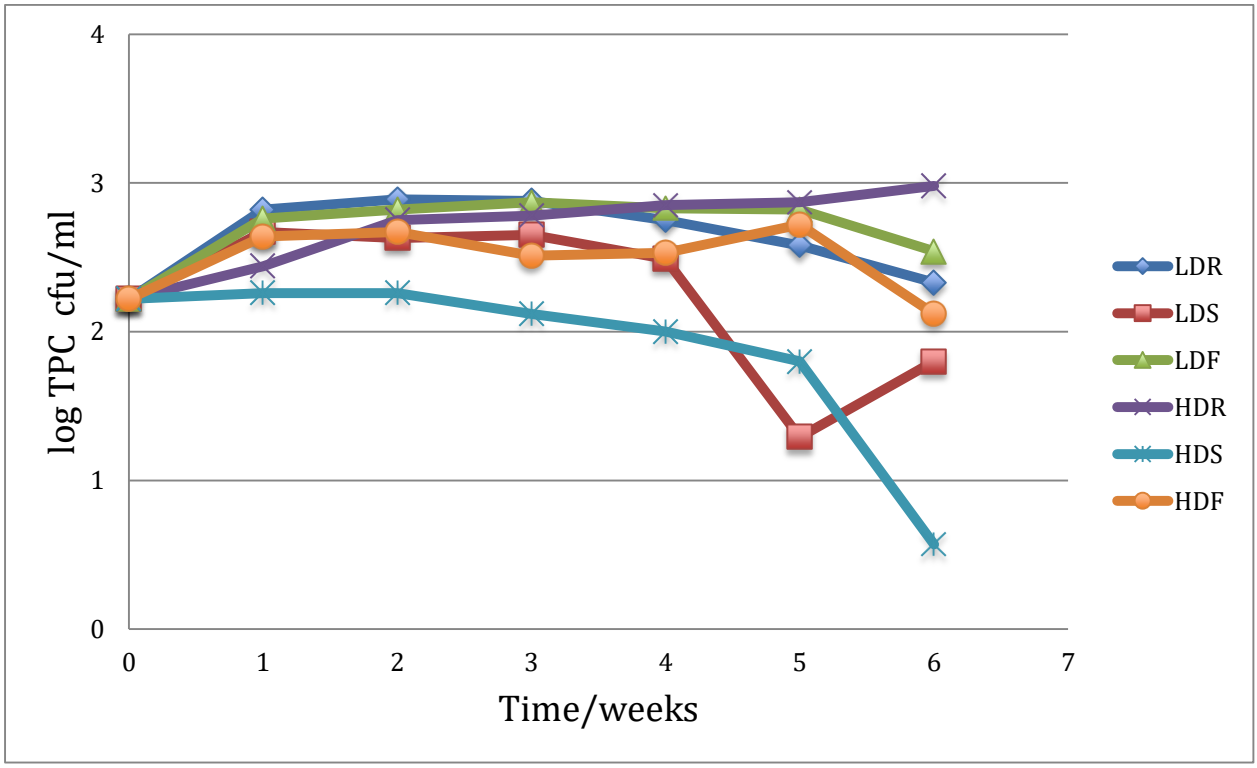


Figure 4.3 Effect of packaging (Polyethylene) type on the Total Plate Count of the stored sachet water

**KEY:**

LDR and HDR water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under room temperature storage
LDS and HDS water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under sunlight exposure
LDF and HDF water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under refrigeration storage

## 4.4. TOTAL COLIFORM COUNT IN THE STORED SACHET WATER

**Table 4.1. Packaging type on the total coliform counts (log cfu/ml)**

| Treatment[a] | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| LDR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LDS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LDF | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| HDR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| HDS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| HDF | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**KEY:**
LDR and HDR water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under room temperature storage
LDS and HDS water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under sunlight exposure
LDF and HDF water samples packaged in Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) respectively under refrigeration storage

# CHAPTER FIVE

## 5.0. DISCUSSION

The changes in pH of the samples in the different packaging types under the different storage conditions over the storage period at 95% confident interval showed no significance differences. However, at the end of the fifth week, there was significant difference ($p<0.05$) with change in pH in the HDS. Eke *et al*. (2012), recorded similar reduction in pH when working on the Production and Quality of 'Dambu-nama' (a Nigerian dried meat product) packaged with LDPE, HDPE, aluminium foil and plastic containers and stored under room temperature for sixteen weeks. Badriah *et al*. (2012), investigated the effects of packaging materials on the quality characteristics of cooked 'Oggtt' and recorded an insignificant reduction in pH during a 60 day storage period at -10 °C ($p\leq0.05$). Standard limit for pH range for water is 6.5-8.5 (Ghana Standards; GS 175-1: 2013). At week 4, the pH for LDF was above the Standard limits for water. However, at week 6, the pH range for all the samples were within the Standard limit.

In most natural waters, pH is controlled by the carbon dioxide–bicarbonate–carbonate equilibrium system and therefore, an increase in carbon dioxide concentration will lower pH whereas a decrease will cause it to rise (WHO, 2003). This may explain why there was general reduction in pH for all samples in the LDPE packages (low barrier for gases) and increased pH in all samples in the HDPE packages (high barrier for gases).

Temperature also affects the equilibrium and pH of water. In pure water, a decrease in pH of about 0.45 occurs as the temperature is raised by 25 °C (WHO, 2003). LDF also recorded the highest average temperature of 29.4 °C in the fourth ($4^{th}$) week. These may have contributed to the low pH for LDF for the $4^{th}$ week.

The temperature range during the investigation ranged from 23.5 °C to 30.2 °C. According to Greenwood *et al*. (2012), mesophiles grow and survive within a temperature range of 20-40

$^{o}$C. Different storage conditions permit growth of different microorganisms (www.boundless.com); for instance, mesophiles at 20-45 °C, psychrophiles at -15-10 °C, and thermophiles at above 45 °C are classified based on the temperature for their growth and survival.

HDPE has a non-polar, linear and relatively simple structure and therefore has a relatively high density (941 to 965 kg/m$^3$) and higher degree of crystallinity (up to 90%). Unlike LDPE, the high degree of crystallinity nature gives it high water vapour and gas barrier properties. The HDPE also has higher softening point, which allows it to withstand high temperatures, such as exposure during steam-sterilization (Bhunia *et al*., 2013). It was, therefore, observed that all samples in HDPE were able to retain relatively higher temperatures compared to samples in LDPE within the same temperature conditions of exposure and storage time. At the end of the sixth week, temperature readings of the samples in the two packaging types were as follows, LDR – 23.47 ± 0.06 °C, LDS 24.83 ± 2.66 °C, LDF – 25.60 ± 1.85 °C, HDR – 26.17± 2.31 °C, HDS – 27.17 ± 0.29 °C and HDF – 24.37 ± 1.67 °C.

The Standard limit for TVC (Total Viable Count) at 37 °C for 48 hours is 500 cfu/ml (log 2.70 cfu/ml) (Ghana Standards; GS 175-1: 2013). At the end of second, third and fourth weeks, the TPC levels for LDR, LDF and HDR were above the Standard limit of log 2.70 cfu/ml. At week five, TPC levels for LDF and HDR were above the Standard limit. Mishra and Mishra (2014), observed TPC levels of 100–200 cfu/100g in the fresh weaning food sample increasing to 500 cfu/100g when the product was packed in HDPE whilst TPC levels of 100–400 cfu/100g increased to 800 cfu/100g when packed in LDPE.

According to Alburelkan (2015), microbial growth is an autocatalytic process and that growth of microorganisms does not occur without the presence of at least one viable cell. The plastic packaging materials of the sachet water may be tight enough to hold water but they are not impenetrable to molecules floating in the air (Ingrid Spilde, 2012).

The TPC level of the sample in the fresh state was log 2.22 ± 0.07 cfu/ml This was significantly different ($p<0.05$), for LDS from the 2nd week to the sixth week of storage, HDR within weeks one and two and for the whole period of storage for HDS except for the 5th week. There was also significant difference between the TPC level for the fresh water and for HDF at weeks two, three and four. During these days, because the sample was freshly treated, the population of the microorganisms that were present may temporarily remain unchanged, as there was no apparent cell division occurring. The cells may be growing in volume or mass and increasing in metabolic activity to adjust to the new environment of the packaging materials used (Todar, 2012).

The rate of the cell division depends on the composition of the growth medium and the conditions of incubation (Todar, 2012). The composition of the packaging materials used and the different storage conditions resulted in the variations of the TPC levels. At the end of the investigation, 67% of the samples in HDPE recorded lower TPC level within an average range of log 0.57 ± 0.98 cfu/ml for sunlight exposure to log 2.12 ± 0.10 cfu/ml for refrigeration condition as compared with samples in LDPE with average TPC level range of log 2.54 ± 0.06 cfu/ml for sunlight exposure to log 1.80 ± 0.17 cfu/ml for refrigeration conditions. The TPC level for HDR was log 2.98 ± 0.03 cfu/ml, which was above the Standard limit of log 2.70 cfu/ml.

From the Temperature Permeability Reference Chart (2016), LDPEs are flexible, can withstand a maximum temperature of 80 $^0$C with oxygen ($O_2$), nitrogen ($N_2$) and carbon dioxide ($CO_2$) permeability of 500, 180 and 2700 cc.-ml/100in$^2$-24hr-Bar respectively. HDPEs on the other hand, are rigid and can withstand temperatures up to 120 $^0$C. They have oxygen ($O_2$), nitrogen ($N_2$) and carbon dioxide ($CO_2$) permeability of 185, 42 and 580 cc.-ml/100in$^2$-24hr-Bar respectively.

Both HDPE and LDPE are translucent but when processed into thin film, LDPE are transparent (Gnanou and Fontanille, 2008). This means that due to the permeability nature of LDPE, it allows better permeation of gases as compared to HDPE. Low temperature resistance as compared with HDPE and the flexibility, LDPE will expand at lower temperatures comparatively. These characteristics of the different packaging material types may have contributed to the different TPC levels observed in this study, a relatively higher TPC levels in LDPE samples than HDPE samples. The changes in TPC levels for the second and third weeks were insignificant ($p>0.05$) for the various conditions. This may be attributed to the possibility that as some of the cells are dying due to secondary metabolites produced by the bacteria cells; the same numbers of cells are dividing (Todar, 2012).

In Figure 4.3, it could be observed that after the third week, most of the TPC levels for the different packaging materials under the different storage conditions declined with the exception of HDR. This may be due the reduction in numbers of the viable cells present (Todar, 2012).

There was a negative correlation (association) between pH and TPC levels for all the packaging types and conditions with storage except for HDR. This implies that as the pH level increases, TPC decreases, and could be as a result of the secondary metabolites being released by the microorganisms thus making the water more basic (Todar, 2012). All the p-values for the different packaging materials under the different storage conditions were, however, insignificant ($p>0.05$). There was a positive association between temperature and TPC level for all the packaging types under the various storage conditions. This means that as temperature increases TPC level increases and vice versa. There was significant difference between temperature and levels of TPC for LDR, LDS and HDF.

Although some of the water samples under the storage conditions in the two packaging types recorded high TPC levels, coliforms were not observed. The results were contrary to works done by Obiri-Danso, *et al* (2003), Olaoye and Onilude (2009), Ezeugwunne, *et al* (2009) and

Akorli (2012). Their investigations into packaged sachet water revealed the presence of total coliforms and faecal coliform (*E. coli*). These contradictions in the investigation findings may be due to the different treatments methods employed by the various companies in the sachet water production (EPA, 2013).

# CHAPTER SIX

## 6.0. CONCLUSIONS AND RECOMMENDATIONS

## 6.1. CONCLUSIONS

The changes in pH of the samples in the different packaging materials under different storage conditions over time showed no significant difference ($p>0.05$). However, at the end of week five (5), there was significant difference in pH change under HDS.

High temperatures resulted in lower pH values as recorded in LDF at the end of week four (4). pH had a negative correlation on TPC levels of the samples in all the different packaging under the different storage conditions with storage time, except for HDR. HDR had a positive correlation in pH with TPC level.

Temperature had a positive correlation on TPC levels of all the samples in the different packaging materials under the different conditions of storage with time. However the correlation was significant with LDR, LDS and HDF; with LDR having a higher correlation coefficient followed by LDS and HDF. It means that temperature and TPC levels had a stronger association in the packaging and condition of storage; followed by LDS and HDF. It can therefore be concluded that, HDPE has a stronger barrier potential to microbial growth and survival than LDPE.

## 6.2. RECOMMENDATIONS

All the packaging materials used for the investigation were of 50micron in thickness. Further works should be done on water packaged in HDPE with different pore size and/or thickness as against microbial growth. This will help establish the appropriate micron (thickness) of HDPE for use in the packaging of sachet water in the country. Sensory analysis of the resultant water samples should also be assessed to determine the acceptability of the water samples after the storage period.

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## APPENDICES

**Appendix 1** Raw data of pH of samples with storage time

| Sample Code | | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|---|
| LDR | A | 7.2 | 7.4 | 7.26 | 7.5 | 6.55 | 7.36 | 7.26 |
| | B | 7.1 | 7.42 | 7.04 | 7.56 | 6.35 | 7.39 | 7.22 |
| | C | 7.08 | 7.37 | 6.95 | 7.33 | 6.46 | 7.38 | 7.34 |
| | MEAN | 7.13 | 7.40 | 7.08 | 7.46 | 6.45 | 7.38 | 7.27 |
| LDS | A | 7.2 | 7.44 | 7.06 | 7.28 | 7.1 | 7.29 | 7.58 |
| | B | 7.1 | 7.42 | 7.02 | 7.17 | 6.95 | 7.4 | 7.12 |
| | C | 7.08 | 7.45 | 7.04 | 7.45 | 6.89 | 7.38 | 7.19 |
| | MEAN | 7.13 | 7.44 | 7.04 | 7.30 | 6.98 | 7.36 | 7.30 |
| LDF | A | 7.2 | 6.74 | 7.06 | 7.31 | 6.32 | 7.37 | 7.28 |
| | B | 7.1 | 7.17 | 7 | 7.45 | 6.42 | 7.26 | 7.36 |
| | C | 7.08 | 7.23 | 7.01 | 7.36 | 6.32 | 7.39 | 7.33 |
| | MEAN | 7.13 | 7.05 | 7.02 | 7.37 | 6.35 | 7.34 | 7.32 |
| HDR | A | 7.2 | 7.38 | 7.01 | 7.49 | 7.01 | 7.2 | 7.23 |
| | B | 7.1 | 7.26 | 7.07 | 7.38 | 7.04 | 7.31 | 7.2 |
| | C | 7.08 | 7.41 | 7.07 | 7.4 | 7.02 | 7.29 | 7.44 |
| | MEAN | 7.13 | 7.35 | 7.05 | 7.42 | 7.02 | 7.27 | 7.29 |
| HDS | A | 7.2 | 7.26 | 7.08 | 7.38 | 6.55 | 7.17 | 7.36 |
| | B | 7.1 | 7.44 | 7.09 | 7.35 | 6.41 | 7.2 | 7.24 |
| | C | 7.08 | 7.38 | 7.06 | 7.44 | 6.45 | 7.2 | 7.39 |
| | MEAN | 7.13 | 7.36 | 7.08 | 7.39 | 6.47 | 7.19 | 7.33 |
| HDF | A | 7.2 | 7.23 | 7.04 | 7.31 | 6.59 | 7.18 | 7.25 |
| | B | 7.1 | 7.06 | 7.02 | 7.34 | 6.52 | 7.13 | 7 |
| | C | 7.08 | 7.26 | 7.05 | 7.48 | 6.5 | 7.16 | 7.33 |
| | MEAN | 7.13 | 7.18 | 7.04 | 7.38 | 6.54 | 7.16 | 7.19 |

**Appendix 2** Raw data of temperature of samples With Storage Time

| Sample Code | | Week 0 (Fresh) | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|---|
| LDR | A | 24.5 | 30 | 30.1 | 26.4 | 28.3 | 25 | 23.4 |
| | B | 24.5 | 27.9 | 29.5 | 26.9 | 28.7 | 25.1 | 23.5 |
| | C | 24.5 | 30.2 | 29.5 | 27.3 | 28.9 | 25.1 | 23.5 |
| | MEAN | 24.5 | 29.4 | 29.7 | 26.9 | 28.6 | 25.1 | 23.5 |
| LDS | A | 24.5 | 30.2 | 29.3 | 29 | 26.2 | 24.7 | 27.9 |
| | B | 24.5 | 29.9 | 29.5 | 29.2 | 26.7 | 24.8 | 23.3 |
| | C | 24.5 | 30.4 | 29.7 | 28.9 | 27.2 | 25 | 23.3 |
| | MEAN | 24.5 | 30.2 | 29.5 | 29.0 | 26.7 | 24.8 | 24.8 |
| LDF | A | 24.5 | 27.4 | 28.9 | 24.7 | 29.5 | 24.7 | 23.5 |
| | B | 24.5 | 27.8 | 29.2 | 25.1 | 29.2 | 24.5 | 27 |
| | C | 24.5 | 27.3 | 29.3 | 24.5 | 29.5 | 24.6 | 26.3 |
| | MEAN | 24.5 | 27.5 | 29.13 | 24.77 | 29.4 | 24.6 | 25.6 |
| HDR | A | 24.5 | 29.2 | 29.5 | 28.8 | 27.1 | 24.9 | 23.5 |
| | B | 24.5 | 30.3 | 28.9 | 28.8 | 27 | 24.8 | 27.5 |
| | C | 24.5 | 29.7 | 29.3 | 28.8 | 27.1 | 24.8 | 27.5 |
| | MEAN | 24.5 | 29.7 | 29.2 | 28.8 | 27.1 | 24.8 | 26.2 |
| HDS | A | 24.5 | 30.1 | 29 | 28.7 | 28.8 | 24.6 | 27.5 |
| | B | 24.5 | 29.6 | 28.8 | 28.7 | 29.1 | 24.6 | 27 |
| | C | 24.5 | 29.7 | 29 | 28.5 | 29.2 | 24.6 | 27 |
| | MEAN | 24.5 | 29.8 | 28.9 | 28.6 | 29.0 | 24.6 | 27.2 |
| HDF | A | 24.5 | 28.3 | 29.2 | 28.4 | 26.9 | 24.8 | 23.4 |
| | B | 24.5 | 27.7 | 29.7 | 28.5 | 27.5 | 24.8 | 23.4 |
| | C | 24.5 | 26.7 | 28.8 | 28.4 | 28 | 24.8 | 26.3 |
| | MEAN | 24.5 | 27.6 | 29.2 | 28.4 | 27.5 | 24.8 | 24.4 |

**Appendix 3** Raw data of TPC & TC of samples with storage time (LDPE)

| Storage Period | Bacteria Type | LDR | | | | LDS | | | | LDF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | Mean | A | B | C | Mean | A | B | C | Mean |
| Week 0 | TPC | 150 | 150 | 200 | 167 | 150 | 150 | 200 | 167 | 150 | 150 | 200 | 167 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 1 | TPC | 500 | 800 | 700 | 667 | 500 | 400 | 500 | 467 | 500 | 600 | 650 | 583 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 2 | TPC | 700 | 850 | 800 | 783 | 450 | 400 | 450 | 433 | 750 | 650 | 600 | 667 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 3 | TPC | 700 | 800 | 750 | 750 | 450 | 400 | 500 | 450 | 800 | 650 | 800 | 750 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 4 | TPC | 500 | 550 | 650 | 567 | 250 | 300 | 400 | 317 | 700 | 600 | 700 | 667 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 5 | TPC | 450 | 300 | 400 | 383 | 150 | 0 | 50 | 67 | 600 | 650 | 750 | 667 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Week 6 | TPC | 200 | 250 | 200 | 217 | 50 | 50 | 100 | 67 | 300 | 350 | 400 | 350 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Appendix 4** Raw data of TPC & TC of samples with storage time (HDPE)

| Storage Period / | Bacteria Type | HDR | | | | HDS | | | | HDF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | MEAN | A | B | C | MEAN | A | B | C | MEAN |
| WEEK 0 (FRESH) | TPC | 150 | 150 | 200 | 167 | 150 | 150 | 200 | 167 | 150 | 150 | 200 | 167 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WEEK 1 | TPC | 200 | 300 | 350 | 283 | 200 | 150 | 200 | 183 | 300 | 500 | 550 | 450 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WEEK 2 | TPC | 550 | 550 | 600 | 567 | 200 | 150 | 200 | 183 | 400 | 500 | 500 | 467 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WEEK 3 | TPC | 550 | 600 | 650 | 600 | 150 | 100 | 150 | 133 | 250 | 400 | 350 | 333 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WEEK 4 | TPC | 650 | 700 | 800 | 717 | 100 | 100 | 100 | 100 | 250 | 450 | 350 | 350 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WEEK 5 | TPC | 650 | 750 | 850 | 750 | 50 | 50 | 100 | 67 | 450 | 650 | 500 | 533 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WEEK 6 | TPC | 950 | 900 | 1000 | 950 | 0 | 0 | 50 | 17 | 150 | 100 | 150 | 133 |
| | TOTAL COLIFORM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Appendix 5** Effects of packaging type on Total Plate Counts (log cfu/ml)* in sachet water before and after 6 weeks of storage

| Treatment[a] | Day 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| LDR | $2.22 \pm 0.07^{Ca}$ | $2.82 \pm 0.10^{Aa}$ | $2.89 \pm 0.04A^{a}$ | $2.88 \pm 0.03^{Aa}$ | $2.75 \pm 0.06^{ABa}$ | $2.58 \pm 0.09^{Bab}$ | $2.33 \pm 0.06^{Cab}$ |
| LDS | $2.22 \pm 0.07^{Aba}$ | $2.67 \pm 0.06^{Aab}$ | $2.63 \pm 0.03^{Ac}$ | $2.65 \pm 0.05^{Abc}$ | $2.49 \pm 0.10^{ABb}$ | $1.29 \pm 1.15^{Bb}$ | $1.80 \pm 0.17^{ABb}$ |
| LDF | $2.22 \pm 0.07^{Ca}$ | $2.76 \pm 0.06^{Aa}$ | $2.82 \pm 0.05A^{ab}$ | $2.87 \pm 0.05^{Aa}$ | $2.83 \pm 0.04^{Aa}$ | $2.82 \pm 0.05^{Aa}$ | $2.54 \pm 0.06^{Bab}$ |
| HDR | $2.22 \pm 0.07^{Da}$ | $2.44 \pm 0.12^{Cbc}$ | $2.75 \pm 0.02^{Bbc}$ | $2.78 \pm 0.04^{Bab}$ | $2.85 \pm 0.05^{ABa}$ | $2.87 \pm 0.06^{ABa}$ | $2.98 \pm 0.03^{Aa}$ |
| HDS | $2.22 \pm 0.07^{Aa}$ | $2.26 \pm 0.07^{Ac}$ | $2.26 \pm 0.07^{Ad}$ | $2.12 \pm 0.10^{Ad}$ | $2.00 \pm 0.00^{Ac}$ | $1.80 \pm 0.17^{Aab}$ | $0.57 \pm 0.98^{Bc}$ |
| HDF | $2.22 \pm 0.07^{Ba}$ | $2.64 \pm 0.14^{Aab}$ | $2.67 \pm 0.06^{Ac}$ | $2.51 \pm 0.10^{Ac}$ | $2.53 \pm 0.13^{Ab}$ | $2.72 \pm 0.08^{Aa}$ | $2.12 \pm 0.10^{Bab}$ |

*Values represent the means ± standard deviations of triplicate measurements.
[a]Low density polyethylene at room temperature (LDR); Low density polyethylene at refrigeration temperature (4 °C; LDF); Low density polyethylene under sunlight (LDS); High density polyethylene at room temperature (HDR); High density polyethylene at refrigeration temperature (4 °C; HDF); High density polyethylene under sunlight (HDS).
[A-C]Means with different superscripts within a row indicate significant differences ($P< 0.05$; Tukey's test).
[a-c]Means with different superscripts within a column indicate significant differences ($P< 0.05$; Tukey's test).

**Appendix 6** Effects of packaging type on pH of sachet water before and after 6 weeks of storage

| **Treatment[a]** | **Day 0** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** | **Week 6** |
|---|---|---|---|---|---|---|---|
| LDR | $2.22 \pm 0.07^{Ca}$ | $2.82 \pm 0.10^{Aa}$ | $2.89 \pm 0.04A^{a}$ | $2.88 \pm 0.03^{Aa}$ | $2.75 \pm 0.06^{ABa}$ | $2.58 \pm 0.09^{Bab}$ | $2.33 \pm 0.06^{Cab}$ |
| LDS | $2.22 \pm 0.07^{Aba}$ | $2.67 \pm 0.06^{Aab}$ | $2.63 \pm 0.03^{Ac}$ | $2.65 \pm 0.05^{Abc}$ | $2.49 \pm 0.10^{ABb}$ | $1.29 \pm 1.15^{Bb}$ | $1.80 \pm 0.17^{ABb}$ |
| LDF | $2.22 \pm 0.07^{Ca}$ | $2.76 \pm 0.06^{Aa}$ | $2.82 \pm 0.05A^{ab}$ | $2.87 \pm 0.05^{Aa}$ | $2.83 \pm 0.04^{Aa}$ | $2.82 \pm 0.05^{Aa}$ | $2.54 \pm 0.06^{Bab}$ |
| HDR | $2.22 \pm 0.07^{Da}$ | $2.44 \pm 0.12^{Cbc}$ | $2.75 \pm 0.02^{Bbc}$ | $2.78 \pm 0.04^{Bab}$ | $2.85 \pm 0.05^{ABa}$ | $2.87 \pm 0.06^{ABa}$ | $2.98 \pm 0.03^{Aa}$ |
| HDS | $2.22 \pm 0.07^{Aa}$ | $2.26 \pm 0.07^{Ac}$ | $2.26 \pm 0.07^{Ad}$ | $2.12 \pm 0.10^{Ad}$ | $2.00 \pm 0.00^{Ac}$ | $1.80 \pm 0.17^{Aab}$ | $0.57 \pm 0.98^{Bc}$ |
| HDF | $2.22 \pm 0.07^{Ba}$ | $2.64 \pm 0.14^{Aab}$ | $2.67 \pm 0.06^{Ac}$ | $2.51 \pm 0.10^{Ac}$ | $2.53 \pm 0.13^{Ab}$ | $2.72 \pm 0.08^{Aa}$ | $2.12 \pm 0.10^{Bab}$ |

*Values represent the means ± standard deviations of triplicate measurements.
[a]Low density polyethylene at room temperature (LDR); Low density polyethylene at refrigeration temperature (4 °C; LDF); Low density polyethylene under sunlight (LDS); High density polyethylene at room temperature (HDR); High density polyethylene at refrigeration temperature (4 °C; HDF); High density polyethylene under sunlight (HDS).
[A-C]Means with different superscripts within a row indicate significant differences ($P< 0.05$; Tukey's test).
[a-c]Means with different superscripts within a column indicate significant differences ($P< 0.05$; Tukey's test).

**Appendix 7** Effects of packaging type on temperature of sachet water before and after 6 weeks of storage

| **Treatment[a]** | **Day 0** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** | **Week 6** |
|---|---|---|---|---|---|---|---|
| LDR | $2.22 \pm 0.07^{Ca}$ | $2.82 \pm 0.10^{Aa}$ | $2.89 \pm 0.04A^{a}$ | $2.88 \pm 0.03^{Aa}$ | $2.75 \pm 0.06^{ABa}$ | $2.58 \pm 0.09^{Bab}$ | $2.33 \pm 0.06^{Cab}$ |
| LDS | $2.22 \pm 0.07^{Aba}$ | $2.67 \pm 0.06^{Aab}$ | $2.63 \pm 0.03^{Ac}$ | $2.65 \pm 0.05^{Abc}$ | $2.49 \pm 0.10^{ABb}$ | $1.29 \pm 1.15^{Bb}$ | $1.80 \pm 0.17^{ABb}$ |
| LDF | $2.22 \pm 0.07^{Ca}$ | $2.76 \pm 0.06^{Aa}$ | $2.82 \pm 0.05A^{ab}$ | $2.87 \pm 0.05^{Aa}$ | $2.83 \pm 0.04^{Aa}$ | $2.82 \pm 0.05^{Aa}$ | $2.54 \pm 0.06^{Bab}$ |
| HDR | $2.22 \pm 0.07^{Da}$ | $2.44 \pm 0.12^{Cbc}$ | $2.75 \pm 0.02^{Bbc}$ | $2.78 \pm 0.04^{Bab}$ | $2.85 \pm 0.05^{ABa}$ | $2.87 \pm 0.06^{ABa}$ | $2.98 \pm 0.03^{Aa}$ |
| HDS | $2.22 \pm 0.07^{Aa}$ | $2.26 \pm 0.07^{Ac}$ | $2.26 \pm 0.07^{Ad}$ | $2.12 \pm 0.10^{Ad}$ | $2.00 \pm 0.00^{Ac}$ | $1.80 \pm 0.17^{Aab}$ | $0.57 \pm 0.98^{Bc}$ |
| HDF | $2.22 \pm 0.07^{Ba}$ | $2.64 \pm 0.14^{Aab}$ | $2.67 \pm 0.06^{Ac}$ | $2.51 \pm 0.10^{Ac}$ | $2.53 \pm 0.13^{Ab}$ | $2.72 \pm 0.08^{Aa}$ | $2.12 \pm 0.10^{Bab}$ |

*Values represent the means ± standard deviations of triplicate measurements.
[a]Low density polyethylene at room temperature (LDR); Low density polyethylene at refrigeration temperature (4 °C; LDF); Low density polyethylene under sunlight (LDS); High density polyethylene at room temperature (HDR); High density polyethylene at refrigeration temperature (4 °C; HDF); High density polyethylene under sunlight (HDS).
[A-C]Means with different superscripts within a row indicate significant differences ($P< 0.05$; Tukey's test).
[a-c]Means with different superscripts within a column indicate significant differences ($P< 0.05$; Tukey's test).

**Appendix 8** Pearson correlation coefficients (*r*) among TPC (log cfu/ml), pH and temperature (°C) for the various package types

| **Treatment**[a] | **TPC - pH** | | | **TPC - Temperature** | |
|---|---|---|---|---|---|
| | ***R*** | ***p*-value**[b] | | ***r*** | ***p*-value**[b] |
| LDR | -0.04 | 0.8468 | | **0.81** | <0.0001 |
| LDS | -0.27 | 0.230 | | **0.59** | 0.005 |
| LDF | -0.14 | 0.551 | | 0.42 | 0.056 |
| HDR | 0.06 | 0.809 | | 0.05 | 0.839 |
| HDS | -0.14 | 0.546 | | 0.16 | 0.494 |
| HDF | -0.09 | 0.683 | | **0.54** | 0.012 |

[a]See Table 4.3 for details
[b] For the null hypothesis (Ho): $r$ = zero.
[C] Bolded $r$ values are significant at $P < 0.05$.