ASSESSMENT OF PHTHALATES MIGRATION IN POLYETHYLENE FOOD PACKAGES: A CASE STUDY IN GHANA

BY

AYAMBA ADONGO ABDUL-MALIK

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL CHEMISTRY DEGREE

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DEPARTMENT OF CHEMISTRY

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Table of Contents

DECLARATION

I hereby declare that this thesis is my own work towards the award of MPhil Chemistry in the Department of Chemistry, University of Ghana, and has neither in part nor in whole been presented for another degree elsewhere.

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Dr. Kwaku Kyeremeh

(Co-supervisor)

DEDICATION

To my family and all loved ones

ACKNOWLEDGEMENTS

I thank God Almighty Allah for His guidance and mercies throughout this work. To prof. Derick Carboo and Dr Kwaku Kyeremeh, thank you for taking time off your busy schedule to supervise my work. To all friends and loved ones, I say thank you for your support and advice.

ABSTRACT

The use of plastics such as polyethylene in the food industry in Ghana as opposed to traditional forms of packaging such as the use of leaves and paper is constantly growing with different designs and compositions. These plastics contain chemicals that are added during the manufacturing process. These chemicals, such as plasticizers, are used to make the plastics flexible but are not covalently bonded to the polymer, hence are able to migrate from the food packages into food when heated or stored for a long term. In this regard, plasticizers such as phthalates from food contact materials have raised many concerns due to suspected carcinogenic effects in humans. Other effects due to the exposure to phthalates include damage to liver, kidney, heart, and lungs as well as adverse effects on reproduction, development and blood clotting.

Five different polyethylene packaging materials including: black polythene bags, plain polyethylene bags/films commonly called take away bags, polyethylene plastic bottles, thick plain polyethylene bags/films and polyethylene food containers were assessed.

Total of four phthalates were soxhlet-extracted and identified by GC-MS. The extent of migration of the phthalates into food was assessed using food simulants (distilled water for aqueous food and olive oil for fatty foods) by varying exposure times and temperatures.

The results indicated that the four phthalates under investigation were all detected in the polyethylene packaging materials. DBP was detected in all the polyethylene samples. DEHP was detected in 20 out of 25 polyethylene samples. The concentrations ranged from not detected to 14.30mg/kg for DEHP, 3.60mg/kg to 15.45mg/kg for DBP and not detected to 5.14mg/kg for DEP. BBP was detected in only one out of the 25 samples analysed with a concentration of

1.43mg/kg. The migrated phthalates from the polyethylene packaging materials into the aqueous food simulant were detected in trace concentrations with average values of 1.68µg/kg for DEHP, 0.60µg/kg for DBP and 0.01µg/kg for DEP. The migrations of these phthalates in the aqueous food simulant were all below the EU specification for phthalate migration in food contact materials. The migration of phthalates in the fatty food simulant however showed very high concentrations of phthalates migration from the polyethylene packaging materials into the olive oil. The migrated concentration of DEHP and DBP ranged from 0.69mg/kg to1.60mg/kg and not detected to 1.43mg/kg respectively. The rate of migration of phthalates from the polyethylene packaging materials into food simulant increased with increasing temperature in both food simulants. The results indicated that 28.13% and 34.38% of the migrated DEHP and DBP in the fatty food simulant exceeded the SML for food contact materials according to the EU regulation. Migrated BBP was however not detected in the fatty food simulant (olive oil).

LIST OF TABLES

LIST OF FIGURES

List of abbreviations

DEHP	Di-2 (ethyl hexyl) phthalate
DEP	Di Ethyl Phthalate
DBP	Dibutyl Phthalate
BBP	Benzylbutyl phthalate
NTP	National Toxicology Program
INCPEN	Industry Council for Research on Packaging and Environment
ICI	Imperial Chemical Industry
PSA	Primary Secondary Amines
GC-MS	Gas Chromatography Mass Spectrometer
BPB	Black Polyethylene Bag
TAB	Take Away Polyethylene Bags/films
PPB	Polyethylene Plastic Bottles
PFC	Polyethylene Food Containers
TPB	Thick Polyethylene Bags/films
g	Gram
mL	Millilitre

mg/L	Milligram per Litre
µg/kg	Microgram per kilogram
µg/L	Microgram per Litre
mg/kg	Milligram per Kilogram
SML	Specific Migration Limit
rpm	Revolutions Per Minute

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 BACKGROUND

The use of packaging to aid the preservation or transportation of food is by no means a modern phenomenon. Broad leaves, animal skins, gourds, hollowed out logs, hard 'skin' fruits and vegetables were used as packaging materials by the earliest civilization [44]. This old form of packaging is still used in Ghana and other parts of the world. For example, plantain leaves are still used to carry food stuff from farms to markets hence serving as packaging materials. In addition, most corn meals in Ghana according to Amoa and Muller are still served to consumers as leaves-packaged ready to eat items [43]. These corn meals include Fante Kenkey (Ntaw) (packaged in dried leaves of *musa paradisiaca*), Fante Kenkey (Dokon Pa) (packaged in dried leaves of *sterculia tragacanta*), Ga kenkey (packaged in dried sheaths of *zea mays*), Fomfom (packaged in fresh toasted leaves of *musa paradisiaca*), Nsiho (packaged in fresh leaves of *musa paradisiaca*), Estwe (packaged in dried leaves of *musa paradisiaca*), Kaafa (packaged in fresh leaves of *thespesia populnea),* Nkyekyera (packaged in dried sheaths of *zea mays* or in fresh leaves of *thespesia populnea*), Aboloo (packaged in fresh leaves of *thespesia populnea*), Sugared Kenkey (packaged in dried leaves of *musa paradisiaca*) and Osino graphic (packaged in dried leaves of *marantochloa cuspidata)* [43].

In the last few decades however, due to social and technological changes such as increased consumption of snacks and take away foods, moves to smaller packs sizes to satisfy smaller households and convenience eating, the packaging of food prior to retail sale or consumption has become increasingly important. Hence different forms and designs of modern packaging are used frequently in our society for packaging different everyday products compared to the traditional forms of packaging [5]. The packaging market has therefore really grown and the expansion of the packaging market can be clearly seen on the shelves of any modern super market by the variety of packaging materials used for various products. These packaging materials include paper and coated-paper products, metal cans, ceramics, glass and plastics.

Since the introduction of plastics in the late 1930s, the use of plastics in the packaging industry has increased at a greater rate than other forms of packaging materials [2]. A diverse range of polymers are used in plastic packaging reflecting the different requirements of modern food packaging. The most common plastic materials found in the food industry include; polyethylene, polypropylene, polyvinylchloride (PVC), vinylide chloride, polyethylene terephthalate (PET) and polystyrene. These packaging materials are able to retain product deterioration, retain the beneficial effects of processing, extend shelf-life and maintain or increase the quality or safety of food. In doing so the packaging provides protection from three (3) major classes of external influences; chemical, biological and physical [20]. Chemical protection minimizes compositional changes triggered by environmental influences such as exposure to gases, moisture or light. Biological protection provides a barrier to microorganisms, insects, rodents and other animals thereby preventing diseases and spoilage. Physical protection on the other hand shield food from mechanical damage and include cushioning against the shock and vibration encountered during distribution [20].

Polyethylene, often called polythene, is the simplest and most inexpensive plastic made by addition polymerization of ethylene. It is probably the packaging material most well known to the consumer and is used in greater volumes in Ghana and other parts of the world. For example, a report by Philip Tice stated that, Western Europe polyethylene plastics represented 39% of standard plastics consumed in the year 2000 and this totaled 11.7 million tonnes with the annual demand growth in the Western Europe predicted to be about 6%. Typically, over 50% of polyethylene plastics are consumed in the food retail and food services sectors. [18]. Polyethylene as a packaging material is divided into three types namely High Density Polyethylene (HDPE), the Low Density Polyethylene (LDPE) and the Low Linear Density Polyethylene (LLDPE).

In Ghana, polyethylene as a packaging material represents a large and constantly growing part of the food industry. Patronage of these polyethylene bags is basically due to convenience, sealability and barrier properties to water. Polyethylene bags have become popular in wrapping all kinds of ready to eat and raw foods such as cooked rice and beans (waakye) and porridge which used to be served in leaves and calabashes respectively. Both ready to eat and raw foods are packaged in these polythene bags regardless of the temperature. Foods such as roasted plantain, roasted corn, porridge, fried plantain and beans (red-red) and other local foods sold on the streets or ‘chop bars’ are now packaged with these polyethylene bags. Kenkey and rice are some of the few foods that are not only cooked at very high temperatures with these polyethylene packaging materials but are also stored in them for longer periods.

Though polyethylene packaging is supposed to make food more convenient and give the food greater safety assurance from microorganisms, biological and chemical changes, it could however present a source of contamination through the migration of pollutants from the packaging material into food which can be deleterious to human health. Thus some chemical compounds incorporated within the polymeric packaging material to improve functionality may interact with food components during processing or storage hence migrating into the food. Once these chemical compounds reached a certain limit may jeopardize food quality and safety. Hence in the selection of polymer to use in food packaging, a number of factors have to be considered including the possibility of migration from the food packaging material into the food. Gosselin and Mondy for example, have reported that polyethylene bags and other plastic packaging materials contain a wide range of potential migrants such as residues from polymerization process, degradation compounds, plasticizers and additives; including lead and cadmium [2].

Most of these chemical compounds such as plasticizers, incorporated in polymer packaging materials are low molecular weight species which are not covalently bonded to the polymers hence are able to migrate from the plastic packaging material into food when heated or stored for a long period [3]. These migratory chemical species such as phthalates have however raised many concerns due to their lower molecular weights and demonstrated carcinogenic effects in rodents [2].

Phthalates are low molecular weight man-made chemical compounds used in the manufacturing of plastics such as polyethylene packaging materials and therefore have the tendency of leaching from the packaging material into the environment or food. Phthalates are used in various other products such as medical devices, personal care products and construction products making it ubiquitous. A study by the European Commission's Joint Research Center (JRC) among European food laboratories on phthalates in food and food packaging materials found more than ten phthalates with Di-2 (ethyl hexyl) Phthalates (DEHP), Di Ethyl Phthalates (DEP), Benzylbutyl phthalates (BBP) and Dibutyl Phthalates (DBP) being the predominant phthalates. Inhalation, intravenous injection tubing and skin absorption are all potential pathways by which these phthalates can get into the body. Phthalate metabolites for instance have been found in the urine of average Americans and other people worldwide [23]. Various researches by National Toxicology Program (NTP) and Breast Cancer & the Environment Research Centers (BCERC) have stated that phthalates are endocrine disrupters and therefore have been linked to adverse reproductive effects in male rodents [47]. In addition, several studies have also shown that phthalates exposure increases the growth of breast cancer cells in vitro [23]. It is therefore incumbent on regulatory institutions with the support of research institutions to enact legislations that would help curb the use of phthalates in plastics produced locally.

1.2 PURPOSE OF STUDY

Chemical contaminants that may migrate and that may affect the safety and quality of food depend on the nature of the packaging material. The constant introduction of different packaging materials and designs has therefore increased the specific hazards that humans are exposed to due to the migration of chemical contaminants such as phthalates from packaging materials to food during heating or storage. In Ghana however, these packaging material are not only used for food storage or transportation but foods such as kenkey are cooked with these packaging materials at very high temperatures for longer periods hence the possibility of increasing the migration rate thereby increasing the specific hazards that human beings are exposed to.

Regulatory authorities and research institutions around the world such as the Food and Drugs Administration (FDA) of the United States and National Toxicology Program (NTP) have carried out several researches on chemical pollutants migration from food contact materials into food and have therefore recognized the need to control such contaminants by enacting legislations. These chemical pollutants such as phthalates are extensively used as additives or catalysts in the manufacturing of plastics hence making it ubiquitous in the environment with several routes of human exposure.

The extensive contamination of the environment by these chemical pollutants has therefore made the analysis of phthalates for example in foods and food contact materials very demanding. In Ghana however, little or no work has been carried out on chemical contaminants migration in food or food contact materials. The purpose of this study is therefore to assess the quality and safety of polyethylene packaging materials used in the Ghanaian food industry in terms of chemical (phthalate) migration.

1.3 HYPOTHESIS

H_0: Polyethylene packaging materials contain migratory phthalates.

H_1: Polyethylene packaging materials do not contain migratory phthalates

H_0: Temperature and exposure times have effect on phthalate migration

H_1: Temperature and exposure times do not have effect on phthalate migration

1.4 AIM

The aim of this study is to assess the level of phthalate migration in polyethylene food packaging materials sold on the Ghanaian market using distilled water and olive oil as food simulants.

1.5 SPECIFIC OBJECTIVES

1. To determine the presence of Di (2-ethyl hexyl) phthalate (DEHP), Benzyl butyl phthalates (BBP), Dibutyl phthalate (DBP) and Diethyl phthalate (DEP) in polyethylene food packages on the Ghanaian market.

2. To quantify DEHP, DBP, BBP and DEP in polyethylene food packages on the Ghanaian market.

3. To identify the trend of migration, if any, of phthalates in polyethylene food packages with respect to temperature and storage times.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 PACKAGING

The goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer desires, maintains food safety and quality, and minimizes environmental impact. Thus packaging protect food from environmental influences such as heat, light, the presence or absence of moisture, oxygen, pressure, enzymes, spurious odors, microorganism, insects, dirt, dust particles, gaseous emissions and so on. The packaging material should therefore be inert and resistant to hazards and not also allow molecular transfer from or to packaging materials. Packaging materials are widely used in the food industry and come with different designs and shapes.

The selection of most appropriate packaging material is essential to maintain the quality and safety of foods. Flexible and rigid plastics such as polyethylene and plastic laminates are the most common materials used for packaging foods [40]. Advances in polymer processing have also enabled the development of plastics that are better suited to different food packaging applications.

2.1.1 FUNCTIONS OF PACKAGING MATERIALS

Unlike traditional packages, which are passive barriers designed to delay the adverse effects of the environment on the food, modern forms of packaging allow packages to interact with the food product and the environment and play a dynamic role in food preservation[36]. The basic functions of food packaging materials therefore include the following;

a) Provide product information

b) prevent physical damage to the product,

c) prevent contamination from microorganism and pollution,

d) protect against dehydration or dampness,

e) Keep the product in peak condition and also help to increase the shelf life of the product.

2.1.2 TYPES OF PACKAGING MATERIALS

There are different designs and construction of food packages that play important roles in determining the shelf life of food products [19]. Selection of the right packaging material and technology therefore maintains product quality and freshness during distribution and storage. Packaging materials that have traditionally been used in food packaging include

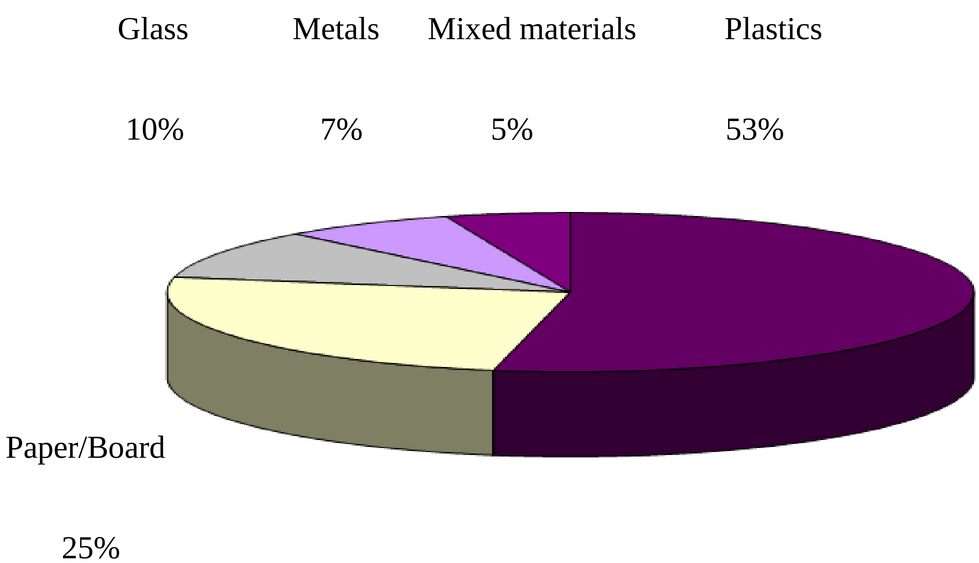
a) Paper and paper board

b) Metal can

c) Glass

d) Plastics

Plastic packaging materials however, compared to paper/board and other packaging materials are the most widely used, with about 53% of all goods packaged in plastics as shown in **fig 2.1** below [19]. These plastic packages contain various components such as polymers, coatings/ink, solvents and adhesives. The polymers of the plastic packages mostly are made up of monomers, plasticizers, catalysts, antioxidants, blocking agents and antislipping agents. Plastics possesses advantages over other packaging materials as they can be made into sheets, different shapes and structures, offering limitless designs of flexibility and easy to carry. They are chemical resistant which is inexpensive and possess wide range of physical and optical properties and offer a wide angle of product design and function. These plastics are very light, low weight and relative strength making it one of the most energy efficient, robust and economic packaging types available. [16].



Source: The Industry Council for Research on Packaging and the Environment, 2001

Fig.2.1. Percentage use of packaging materials in all packaged products

2.1.3 TYPES OF PLASTIC PACKAGING MATERIALS

Plastics are made by condensation polymerization (Polycondensation) or addition polymerization (polyaddition) of monomer units. In polycondensation, the polymer chain grows by condensation reactions between molecules and is accompanied by formation of low molecular weight byproducts such as water and methanol [14]. Polycondensation involves monomers with at least 2 functional groups such as alcohol, amine or carboxylic groups. On the other hand, polyaddition involve the growing of the polymer chain by addition reaction in which two or more molecules are combined to form a larger molecule without liberating any by-product. These reactions involve unsaturated monomers, that is double bonds or triple bonds are broken to link monomer chains [14].

Plastics are categorized into two major types namely thermosets and thermoplastics. Thermosets are polymers that solidify and become irreversible when heated hence cannot be remolded. Thermoplastics on the other hand are polymers that when expose to heat can be reversed to its original shape. Thermoplastics are therefore mostly used for food packages because it can easily be shaped and molded into various products such as bottles, plastic films, etc. [20].

A wide range of plastics are being used as materials for packaging including polyolefin, polyester, polystyrene, polyvinyl chloride, polyamide, polyvinylidene and ethylene vinyl alcohol. Polyolefin which is a collective term for polyethylene and polypropylene is common and widely used in the food industry.

According to K. Marsh and B. Bugusu, advances in food processing and food packaging play a primary role in keeping the food supply chain safe [20]. Packaging maintains the benefits of food processing after the process is complete, enabling the food to travel safely for long distances from their point of origin and still be wholesome at the time of consumption.

2.2 POLYETHYLENE

Polyethylene, a thermoplastic, is the principal member of the polyolefin class of polymers. The discovery of polyethylene in 1933 by scientist of the Imperial Chemical Industry (ICI) laboratory in U. K was by an accident. They found out that when ethylene and benzaldehyde was subjected to high pressure, a wax-like polymeric substance was formed. This discovery led to a free radical processes operating at high pressures and high temperatures to produce a branched ethylene polymers now called low density Polyethylene (LDPE) in the late 1930s[1].

2.2.1 TYPES OF POLYETHYLENE

Polyethylene manufacturing is usually categorized into high pressure operations for Low Density Polyethylene (LDPE) and Low pressure operations for High Density Polyethylene (HDPE). The third type of polyethylene is the Low Linear Density Polyethylene (LLDPE).

The polyethylene plastics produced today are based on a family of polymers derived from ethylene and various α-olefin co-monomers, including 1-propene (propylene), 1-butene, 1-hexene, and 1-octene. They are the most widely used plastics for commodity packaging [18]. Other polyethylene co-polymers are manufactured with co-monomers such as vinyl acetate, producing the ethylene vinyl acetates, and various acrylics, producing, for example, the co-polymers known as the ionomers. [18]

The LDPE is flexible, strong, tough, easy to seal and resistant to moisture. Basically bread and frozen food bags, flexible lids, squeezable food bottles are examples of LDPE. These types of polyethylene are produced by autoclave or tubular rector technology at a very high pressure [up to 45,000 psi]. The resultant polymer is highly branched resulting in its low density and unique physical properties. Vinyl acetate can be added to produce copolymer product that can have increased clarity and flexibility [22]. The LDPE packages are slightly cloudy, have high tensile strength, good vapour barrier property but with high gas transmission.

The LLDPE is typically made by using a transitional metal catalyst in a gas-phase rector at low pressure relative to the high pressure used in the manufacture of LDPE. Co-monomers such as butene, hexene or octene are added with ethylene to create linear polymer chains with short chain branches and low densities [22]. The LLDPE has water vapour and gas permeation properties similar to LDPE films. It however has about 75% higher tensile strength, 50% high elongation at break, better impact and puncture than the LDPE.

High Density Polyethylene can be polymerized by using slurry, solutions or gas-phase rector technologies. The HDPE manufacturing processes also uses transition metal catalysts to make linear, polymer chains with less branching than LLDPE. Butene, hexene, and octene are typical HDPE co-monomers. Polymerizing ethylene without any co-monomer produces HDPE homopolymer. The resulting products exhibit the highest density and crystallinity in polyethylene family as shown below [22]. The HDPE packages are translucent, stiffer and have lower elongation at break than the LDPE. The water vapour and gas permeation of the HDPE is slightly higher than LDPE.

Table.2.1. Types of polyethylene materials

P.E Polymer	Density /g/cm^3	Degree of crystallinity, %	Melting point range, ^{0}c	Molecular weight, Daltons
LDPE	0.915-0.940	45-55	105-115	10000-50000
LLDPE	0.915-0.926	30-45	112-124	50000-200000
HDPE	0.940-0.970	70-90	120-130	Up to 250000

The principal physical property making the differences between the polyethylene types is the density. The differences in density are basically due to differences in the degree of crystallinity, which also influences the plastics melting point ranges according to Brandy et al [18]. Typical values for these parameters are listed in **table 2.1.**

Most of these polyethylene polymers are known to contain a wide range of additives which are necessary for processing and improving the quality of the final product in terms of durability, flexibility and appearance [16]. Addition of plasticizers for instance improves the flexibility and workability of some rigid polymers. These plasticizers such as phthalates are low molecular weight additives which do not covalently bond with the polymer chain and therefore could potentially migrate into food during heating or storage [3].

Most food stuffs come into contact with these packaging materials during cooking or food handling hence there is the possibility of these additives to contaminant foodstuff by migrating from the plastic packages into the food.

Most of these pollutants which are either organic compounds such as phthalates, adhesives, coatings/inks, monomers or inorganic compounds such as catalyst, lead and cadmium, which may migrate during heating or storage, are toxic to human health. Hence, one of the several forms of human exposure to toxic chemicals can occur from packaging materials and other materials in contact with food due to migration of the contaminants from the packaging material into the foodstuff during heating or storage [4].

Food stored in these plastic packaging materials or cooked with these plastic packaging materials can therefore get contaminated as a result of migration of additives such as plasticizers and stabilizers into the food [17].

2.3 FACTORS THAT CONTROL MIGRATION PROCESS

Migration of contaminants from packaging materials into food is described as a diffusion process that can be subjected to the laws of physics and chemistry [5]. The diffusion of substances from polymers is a very complex process since it depends on several parameters such as;

a) Concentration of substances in packaging film and food
b) Nature of the food
c) Temperature
d) The time period over which duration of contact occurs.

The process of chemical migration can be divided into four (4) major steps; diffusion of chemical compounds through the polymers, desorption of the diffused molecules from the polymer surface, sorption of the compounds at the plastic-food interface and sorption of the compounds in the food [5]. The rate of migration of contaminants from the packaging material into the food decreases with the following;

a) Higher molecular weight substance in the packaging material.
b) Low diffusivity (inert) packaging material
c) Presence of a barrier layer.

There are a wide range of migratory chemicals that have the tendency to migrate from a packaging material such as plastics into food. Some of these migratory chemicals include;

i. Phthalates
ii. Bisphenol A
iii. Perfluorinated compounds (PFC’s)
iv. Epoxidised soybean oil (ESBO)

v. Semicarbazide

vi. Acrylonitrile

vii. Vinyl chloride.

All these chemicals when migrated into food and consumed are toxic to human health at certain concentrations. The use of various forms of plastics during cooking or food storage has raised many concerns in many countries especially the EU over the past three decades [2]. A wide range of these migrants such as plasticizers used in the plastics demonstrated carcinogenic effects in rodents and potential estrogenic effect in humans in a study conducted by NTP Technical Report Series [2].

Monitoring the progress of chemical contaminant migration during heating or storage in a homogeneous liquid or semi-solid would therefore be of immense benefit to public health and legislation.

2.4 INTRODUCTION TO PHTHALATES

Humans are frequently exposed to industrial chemicals directly or indirectly through sources such as food, cosmetics, air, water or pharmaceutical products. Many of these chemicals are toxic at certain concentrations and under certain conditions of exposure. The risk of these industrial chemicals can be estimated by understanding the toxicity and nature of exposures of these chemicals. The toxicity of these chemicals can be influenced by the route of exposure such as through ingestion, inhalation, absorption through the skin and intravenous administration. Some other actors such as timing, duration and pattern of exposure are also important factors that determine the toxic impact of the chemical. Chemicals that do not persist for a long period of time may still pose a problem if the duration of exposure is prolonged. These groups of chemicals are ubiquitous in nature and collectively referred to as phthalates which are produced in millions of tonnes annually. [7]. These compounds found in many poly-based products such as plastics are potential migrants when they come into contact with liquid or solid surrounding media such as food due to their relatively low molecular weight. The lipophilic nature of these esters found in food grade plastics favours their migration into oils and the food fat components [24].

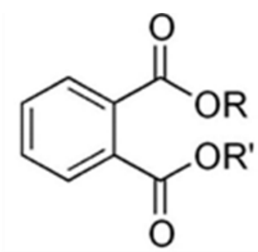
A limited number of studies on humans and a wealth of data on animal studies show that some of these phthalates such as di-(2-ethyhexyl) phthalate are toxic and can impair reproduction and development, alter liver and kidney functions, damage the heart and lungs and also affect blood clotting [24]. The adverse effects of most of these phthalates to human health are well known and concern several aspects of human life, from reproductive and endocrine system to respiratory and dermatological problems. The results of toxicological studies of these phthalates lead to the prohibition of the addition of some of these phthalates in the preparation of plastics intended for toys and for direct food contact wrapping materials in 1999 [24]. A lot of countries especially from the European Union (EU) have come out with regulations to help regulate and monitor the amount of these plasticizers that are added to direct food contact materials such as food packages.

2.4.1 PHTHALATES

Phthalates are a family of synthetic compounds used in the manufacture of plastics including polyethylene and are often called plasticizers [29]. They represent a broad chemical family of diesters generated from alcohols and phthalic anhydride. Phthalates are therefore 1, 2-benzenedicarboxylic acid esters [30]. Phthalates have a wide range of characteristics depending on the chemical structure of the phthalate. The common characteristics of these phthalates however include the following;

a) Soluble in oil
b) Insoluble in water
c) Able to resist high temperature
d) Degrade under exposure to sunlight
e) Metabolized under aerobic microbial activity

Phthalates in their pure form are usually clear liquids, some with faint sweet odors and some with faint yellow colors [29]. The general basic chemical structure of phthalates (R and R' = C_nH_{2n+1}) is shown below.

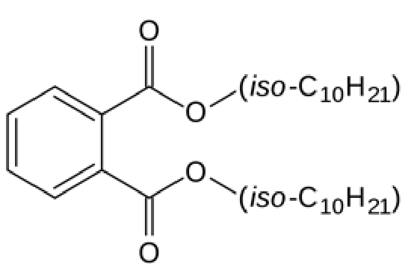


Phthalate diesters simply called phthalates are widely used as plasticizers to increase the flexibility of plastics that are used in the manufacturing of kitchen utensils and food containers [6]. These phthalates are not chemically bonded to the polymeric matrix, which may allow the migration of these chemicals into food substances [6]. These phthalates are not only used in food packages but are also used in medical devices, flooring and wall covering furniture, toys, car interior, paints, printing ink, adhesives, cables, wires and hoses. Human beings are therefore exposed to these phthalates in several ways such as through food, cosmetics, air and pharmaceuticals [21].

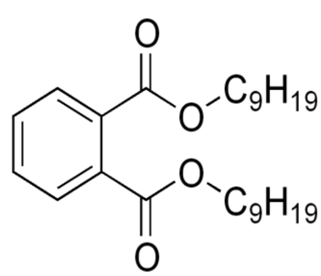
Some of the phthalates commonly used in the manufacturing processes of plastics and other packaging materials include:

i. BBP: benzyl butyl phthalate
ii. DBP: di-butyl phthalate
iii. DEHP: di-(2-ethylhexyl) phthalate
iv. DEP: diethyl phthalate
v. DiBP: di-isobutyl phthalate
vi. DiDP: di-isodecyl phthalate
vii. DiNP: di-isononyl phthalate
viii. DMP: di-methyl phthalate
ix. DnHP: di-n- hexyl phthalate
x. DnOP: di-n-octylphthalate

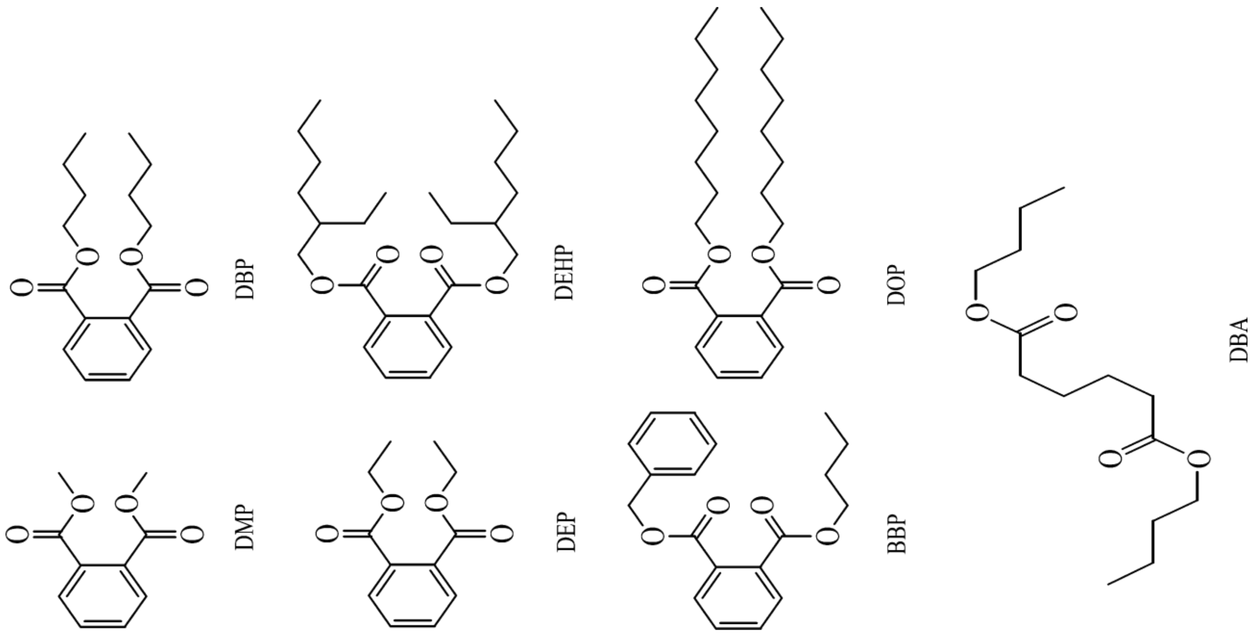
The most widely used phthalates in plastics and other packaging materials in manufacturing processes include the following [25]; di-(2-ethyl hexyl) phthalates (DEHP), dibutyl phthalates (DBP), benzyl butyl phthalates (BBP), di-n-octylphthalate (DOP). The structures of these common phthalates are shown below



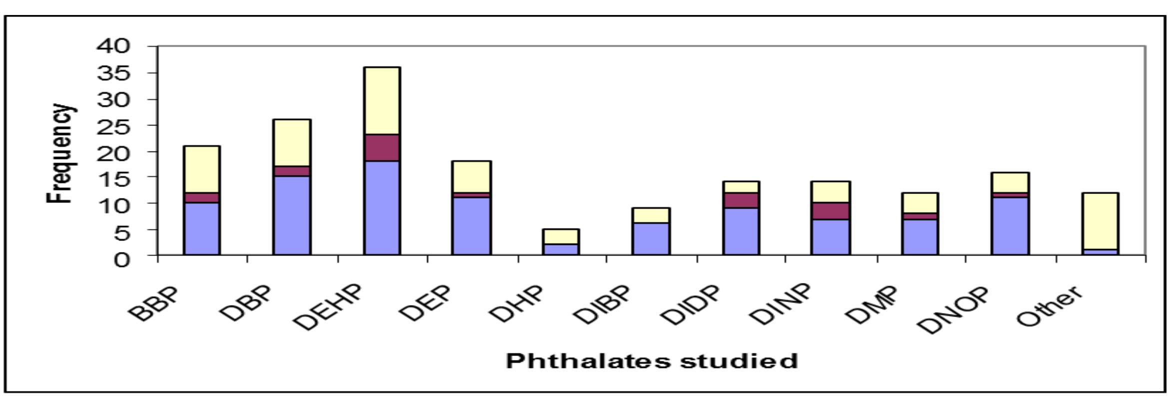
DIDP



DiNP



Di 2-ethylhexyl phthalate, DEHP accounts for about 50% of the world production of phthalates. It is the most frequently detected phthalate. The second and third most frequently analysed phthalates in food and food packages are di Butyl Phthalate, DBP and Benzyl Butyl Phthalate, BBP respectively [25]. A survey conducted by European commission's Joint Research Centre (JRC) in 2007 among European food control laboratories on phthalate determination indicated the frequency of analysis of phthalates in food as shown in **fig 2.2** [25].



Blue: Compiled results of JRC survey
Purple: Compiled results of German survey
Yellow: Compilation of data from literature

Fig.2.2. Frequency of analysis of individual phthalates in foods

2.4.2 SOURCES OF PHTHALATES EXPOSURE

Phthalates are used in a variety of industrial and consumer applications for more than 50 years. They are the most commonly used plasticizers worldwide [30]. Research on both animals and humans demonstrate that exposure may occur throughout the life span, from the developing fetus through early infancy, childhood and beyond [32]. This is because phthalates are constantly and continuously released into the environment by direct release, migration, evaporation, leaching, and abrasion of and from the products they are used in hence making it ubiquitous [32]. Children however are exposed to higher phthalate doses than adults with foodstuff being the major source of exposure, particularly the long chain phthalates such as DEHP. An additional exposure route for young children after food is through mouthing toys, children articles and other products containing phthalates [32].

These chemicals are of concern due to both their widespread use as well as documented toxic effects measured in vitro, in vivo and in epidemiological studies. For example, studies conducted by the center for disease control and prevention have detected metabolites of DEHP and DEP in the majority of U.S population [33]. A case control study conducted in northern Mexico by Lopez-carrillo L. et al also found significantly higher levels of monoethyl phthalate (MEP), a metabolite of DEP in breast cancer cases compared to controls [33].

The long chain phthalates are mostly found in building and construction materials, cables, flooring, clothing, furnishing, car interiors, toys and food contact materials such as food packages. The short chain phthalates such as DEP and BBP on the other hand are used in applications such as personal care products, paints, adhesives and enteric-coated tablets [30]. A research study by Colacino et al. reported that high molecular weight phthalates has been weakly associated with early breast and pubic hair development whilst exposure to low molecular weight phthalates was weakly associated with later breast and pubic hair development [33].

These phthalates exposure is considered harmful to human health due to its toxicological studies in animals which demonstrated considerable adverse health effects [31]. These phthalates can enter the human body through ingestion, inhalation and dermal absorption. Ingestion is a primary pathway of phthalate exposure, especially those in food packages [31]. Food as a major source of phthalate exposure can get contaminated through processing, handling, transportation, packaging and storage. The intake of enteric-coated tablets and the infusion of blood contained in PVC blood bags are major sources of phthalate exposure in medical devices [33].

Phthalates when consumed rapidly hydrolyzed to the monoesters and then metabolized and excreted with urine or feces. Hence the best method for monitoring phthalate exposure in human is by urine analysis [33].

2.4.3 PHTHALATES IN FOOD

Food is a major source of exposure to phthalates [26]. Data on phthalate analysis in food unlike other contaminants is however very limited even though analysis of phthalates in food began about four (4) decades ago according to Cerbulis et al. due to challenges in the method of analysis such as high blank levels of phthalates caused by laboratory environments [27]. Migration from food packaging materials plasticized with phthalates is an important source of phthalates in foods when in contact with the packages [27]. It is therefore important to monitor the levels of phthalates in various kinds of packaged foods to provide data for human exposure assessment since a wide range of phthalates, especially DEHP, have been reported in high concentrations in some foodstuff such as milk, cream and drinks than would have been found in those foods [28]. This is an indication of food contamination.

Phthalate levels in foods based on limited data are highly variable suggesting sporadic contamination which may occur in the source or original collection of the food, in the packaging or processing prior to market or in the preparation leading to consumption. Fatty foods such as dairy products, meats and vegetable oils mostly record higher concentrations of phthalates due to their lipophilic character [26].

Phthalates such as BBP, DEP and DBP have all been detected in infant formulas but in low concentrations compared to DEHP in a survey conducted by the Ministry of Agriculture, fisheries and food (MAFF) in the UK in the mid-1990s. In addition to the infant formulas, these phthalates were also determined in human milk, fresh and canned fish, meat and poultry but in very low concentrations. The survey however did not identify the sources of contamination of these phthalates but suggested that the source of contamination might not only be from the packaging materials used but could also be from other sources since some of the infant formulas in cans were also contaminated [27].

2.4.4 HEALTH EFFECTS OF PHTHALATES

Phthalates are ubiquitous and display a wide variety of toxic effects in animal studies following chronic exposure or even after short-term exposures in particularly vulnerable organisms. These effects include damage to the liver, kidney, heart, and lungs as well as adverse effects on reproduction, development, and blood clotting [7]. Health effects caused as a result of exposure to phthalates differ among the various compounds and depend on the timing and size of the dose. Soharu in a study on the exposure effects of phthalates on humans reported that young and developing organisms are more vulnerable to phthalates exposure than adults with the male reproductive tract being the most sensitive endpoint [7].

The monoester metabolite of some of the parent phthalates compounds such as BBP are thought to be responsible for the reproduction and development effects of phthalates. Some of these effects include decreased fertility in females, fetal defects, reduced survival of offspring, birth defects and altered hormone levels. In males, BBP causes reproductive malformation in infants including altered hormone levels, testicular atrophy, reduced sperm production and motility, sertoli cell damage and leydig cell tumors [7]. BBP also increases the incidence of eczema and rhinitis in children with increased concentration of BBP in house dust. Prenatal exposure of rats to BBP resulted in teratogenic effects in offspring that included skeletal malformations, increased incidence of cleft palate and decreased number of live fetuses at birth. The phthalate, BBP was classified as a “class C”, a possible human carcinogen by U.S. E.P.A based on leukemia in female rats [29].

Phthalates such as DBP, DEHP and DEP causes increase in liver weight which affect liver functions and alter liver enzymes in rodents. Some of these liver changes in rodents are thought not to be relevant to humans. DEHP causes human liver effects such as cholestasis in which bile excretion is impaired as a result of DEHP exposure. The effects of DEHP in male include altered zinc concentrations, testicular atrophy and infertility. Increase in the incidence of asthma in children was associated with increased levels of DEHP concentrations in house dust. Increased exposure of some infants to DEHP from medical devices was also associated with cholestasis (reduced bile flow) and unusual lung disorders [29].

A study by a Swedish national chemical inspectorate on DEHP in 2001 for E. U. concluded in its review that "the effects on testis, fertility and development observed in different animal species and at relatively low levels are considered to be relevant to humans." [7].

As a result of the toxicity of some of these phthalates, scientist and regulatory bodies over the past decades have become more concerned about consumer protection from possible food toxicity caused by the migration of these compounds from food contact materials. Regulatory bodies of various countries such as Canada, U.S.A and some E.U countries have therefore formulated guidelines and regulations for testing such migrants. The testing is done to regulate the specific amount of migrant such as phthalates that should be contained in a packaging material that is in contact with food. [26]. The European Food Safety Authority(EFSA) for example have established the tolerable daily intake (TDI) for phthalates at 0.01 mg/kg body weight per day for DBP, 0.5mg/kg body weight per day for BBP and 0.05mg/kg body weight per day for DEHP based on toxicological studies.[6].

The Food and Drugs Authority in the U.S.A have also regulated the amount of phthalates that should be contained in drug delivery systems, packaging and medical equipments according to the Food and Drugs administration [26]. Phthalates such as DEHP, DBP and BBP however have been banned in children's toys and some child care articles intended to facilitate feeding, sucking and teething due to its toxicity under the consumer product safety improvement Act in the U.S.A. DEHP and DBP are also listed as hazardous pollutants under the clean water Act in the U.S. environmental protection agency 2012b [26].

2.4.5 ALTERNATIVES TO PHTHALATES

As a result of the epidemiological human health impact of phthalates such as reproductive abnormalities and developmental effects in animals in doses that are comparable to that expose by humans, scientist and regulatory institutions are researching to finding alternatives to theses phthalates as plasticizers [34].

A number of substances such as citrates, sebacates, adipates and phosphates are identified as possible alternatives to phthalates as plasticizers. These chemicals are therefore being researched into to enable chemical industries use these chemicals as substitutes for phthalates as plasticizers in products such as medical devices, toys, food packages etc. Not much work however has been done on these chemicals to ascertain its effects on human health if used as alternatives to phthalates [34].

Another alternative been sought for by scientist to replace phthalates is to use plastics or products that do not require the application of plasticizers such as phthalates. Products such as polyurethane, polyethene (HDPE), ethylene vinyl acetate require fewer and less harmful additives compared to products of polyvinyl chloride (PVC) [34].

CHAPTER THREE

3 MATERIALS AND METHOD

3.1 CHEMICALS AND REAGENTS

Ethyl acetate (analytical grade) was purchased from Paskem Fine Chemical Industries (India). The ethyl acetate was redistilled before use. Acetone was purchased from Riedel-de Haen in Germany. Acetonitrile and n-Hexane (pesticide grades) were purchased from VWR, PROLAB (France), formic acid was purchased from sigma Aldrich in Germany. All phthalates standards; Di-(2-ethylhexyl) phthalate [99.8%], Butyl benzyl phthalate [99.7%], Diethyl phthalate [99.9%] and Dibutyl phthalate [99.8%] were purchased from Wako Pure Chemical Industries Ltd, Japan, Magnesium Sulphate and primary secondary amines (PSA) were purchased from Sigma Aldrich in Germany. Distilled water was obtained from the water distillation plant of the chemistry laboratory of Ghana Standards Authority and Olive oil in glass bottles were purchased from a local market.

All glass ware used were washed with detergent, rinsed thoroughly with distilled water and dried in an oven. The dried glass wares were rinsed thoroughly with n-Hexane and acetone before each use. All plastic laboratory materials were totally avoided to minimize contamination

3.2 SAMPLING

Polyethylene samples were purchased from local markets within Accra in the Greater Accra region of Ghana. The product specification mark for the polyethylene food containers and polyethylene drink bottles was looked out for before purchase. Five different categories of the polyethylene samples namely black polythene bags, plain polyethylene bags/films commonly called take away bags, polyethylene plastic bottles, thick plain polyethylene bags/films and polyethylene food containers were purchased from the various markets. These five categories of samples are shown in **fig3.1** below. Five samples each of these five different packaging materials of different brands were bought from the various markets for the phthalates assessment. Each batch of samples was stored separately to avoid cross contamination.

Black polyethylene bags

Polyethylene Bottles

Take away bags

Thick Plain Polyethylene Bags

Plastic Food containers

Fig.3.1. Categories of common polyethylene food packages

3.2.1 IDENTIFICATION OF PLASTIC SAMPLES

The identity of the polyethylene food containers and the polyethylene bottles in each batch of samples was confirmed in the laboratory after identifying the product in the market by the product identification mark. The identities of all the samples were confirmed by conducting elemental identification analysis using both flame test and melting point determination test. This also helped in the sample identification and categorization as HDPE or LDPE base on the melting points as shown in appendix 1A

3.2.1.1 MELTING POINT TEST

A very small portion of each of the samples was carefully and accurately placed into a capillary tube and inserted into the Aluminium sample block of the Stuart melting point apparatus SMP30 and the melting point of each sample determined. The plateau temperature of each sample was set at 95°C and the ramp rate set at 5°C. The melting point of each sample was carefully taken and recorded [35]. The average melting point of each of the various batches of samples for each category was then calculated and recorded as shown in appendix 1B

3.2.1.2 FLAME TEST

About 0.1g of each sample was accurately weighed and placed on a clean spatula. The spatula was gently warmed on a Bunsen burner flame to fume. The heating continued until the sample began to burn with a yellow tip and a blue base. The sample also continued to burn when removed from the flame indicating that the sample was polyethylene [22].

3.3 POLYETHYLENE SAMPLE EXTRACTION

Each of the five samples of the five categories of polyethylene packaging materials purchased in the various markets were subsampled and cut into 3 x 3cm pieces and thoroughly mixed to give a representative sample. About 2.0g each of the sample was accurately weighed into a new extraction thimble and extracted in a soxhlet apparatus [Gallen Kjeldahl] using 200 mL ethyl acetate in a 500ml round bottom flask at the boiling point of ethyl acetate for six (6) hours 30 minutes. The new thimbles each was rinsed with n-hexane before used. Each batch of samples consisted of a blank sample and a spiked sample for quality checks. The samples after the required time of extraction were allowed to attain room temperature and then concentrated to dryness and reconstituted with 2mL ethyl acetate. Each sample was then picked into a vial after ultra-sonication for 1 minute and then analysed with a GC-MS equipped with an auto sampler for qualitative identification and quantification of the phthalates present. To check the effectiveness of the extraction, each of the samples was extracted for the second and third times using fresh ethyl acetate and analysed with the GC-MS.

3.4 MIGRATION TEST USING DISTILLED WATER

To evaluate the migration of phthalates into aqueous foods, ultrapure water was used as food simulants according to the European commission regulation No.10/2011.

This study tested five different categories of polyethylene packaging materials commonly used in the food industry and sold on the Ghanaian markets. These food packages included three different types of polyethylene bags/films, plastic bottles and plastic food containers. All these food packages studied were of different brands collected from different local markets within Accra. Each of the samples was cut into 3 x3 cm pieces similar to that used for the soxhlet extraction. About 2.0g each of the samples was accurately weight and immersed in 100ml distilled water in a clean 250mL beaker as aqueous food simulant. The samples were heated for 30mins, 1hr, 2hrs and 4hrs at temperatures of 20^{o}C, 40^{o}C and 80^{o}C using a water bath in order to control the temperatures. Another set of samples were accurately weighed into beakers of 100mL distilled water and put in a fridge at a temperature of 5^{o}C for 30 mins, 1hr, 2hrs and 4hrs with the maximum and minimum temperatures set at 9^oC and 1^oC [$\pm 4^0C$] respectively to regulate the temperature of the fridge. The temperature conditions and exposure times were chosen based on conditions that are commonly used in everyday life, where the higher temperature is used for cooking food, the medium temperature is for thawing food or handling hot foods and the lower temperature is for frozen foods. The samples after the time allocated and temperatures assigned, were removed, allowed to cool and the aqueous solution for each sample was extracted into 50ml ethyl acetate using 250mL separatory funnel. The extraction with the ethyl acetate was repeated two more times and the organic layers combined and dried over 1g of magnesium sulfate, filtered and evaporated in vacuo using rotary evaporator. The residue was reconstituted with 2mL of pure ethyl acetate and subjected to a GC-MS analysis after ultra-sonication for one minute.

The analysis of each batch of samples contained a sample blank which contained the distilled water without any packaging material. The sample blanks and spiked samples were analysed as samples alongside the plastic packaging material samples to check cross contamination and the efficiency of the method.

3.5 MIGRATION TEST USING OLIVE OIL

The analysis of phthalates migration from polyethylene packaging materials into olive oil as a fatty food simulant was carried out using two out of the five categories of the polyethylene food packages commonly found in the food industry according to European commission regulation No. 10/2011. These are the take away polyethylene bags (TAB) and the polyethylene food containers (PFC). This analysis was carried out with extreme care due to the ubiquitous nature of phthalates and the high affinity for fats by phthalates hence the tendency of cross contamination of the samples especially the sample blanks [45]. Each sample was analysed in duplicates.

Each of the two samples was cut into 3x3cm pieces similar to that used in the soxhlet extraction and 2.0g each accurately weighed into clean 100mL beakers containing 20mL of olive oil. The samples were then heated in a water bath for 30minutes, 1 hour, 2 hours and 4 hours at constant temperatures of 20°C, 40°C and 80°C whilst another set of samples were kept in a fridge at a temperature of 5°C for the same exposure times. Each batch contained a sample blank. Some of the samples were spiked by standard addition and taken through the analytical process alongside the test materials similar to that carried out in the aqueous food simulant above. 10mL acetonitrile was added to each of the samples including the sample blanks and the spiked samples after the required temperature and exposure times were reached and the samples allowed to attain room temperature. It was then vortex for 1 minute and centrifuge for 5 minutes at 3000rpm. The samples were left over night in a fridge to freeze. The samples were again centrifuge for 5 minutes at 3000rpm after freezing and 6mL each of the samples picked for cleanup.

For the cleanup, 150mg of PSA was accurately weighed into a glass centrifuge tube and 900mg $MgSO_4$ added. The 6mL extract of the acetonitrile layer was then added and centrifuge for 5 minutes at 3000rpm after 1 minute of vortex. 4mL of the clean sample was carefully taken into a pear shaped flask and 40μL of 1% formic acid in acetonitrile added. The samples were then concentrated to dryness using a rotary evaporator and reconstituted with 2mL pure ethyl acetate. The 2mL ethyl acetate extract was then pick into vials after ultra-sonication for 1 minute and analysed with the GC-MS using the same settings as described below.

3.6 PREPARATION OF PHTHALATE STANDARDS

A bulk mixed phthalates standard of 100mg/L was prepared from the individual standards purchased from wako pure chemical industries Ltd in Japan. This was done by accurately weighing 0.01g (10mg) of each of the stock standards into 20mL clean dried beakers. The standards were then dissolved in about 10mL ethyl acetate in each of the beakers and combined into a 100mL volumetric flask. The beakers were rinsed thoroughly with ethyl acetate and added to the 100mL volumetric flask and made up to the 100mL mark with ethyl acetate. A mixed working phthalates standard of 100µg/L was then prepared from the bulk phthalates standard by taking 0.1mL of the stock standards into a clean 100mL volumetric flask and topping up with ethyl acetate to the 100mL mark. Calibration standards of 1µg/L, 5µg/L, 10µg/L and 20µg/L for the migration test were prepared from the mixed working standard by taking 0.2mL, 1mL, 2mL and 4mL respectively from the working phthalates standard and making it up to the 20mL mark of the volumetric flask for each of the calibration standards. Calibration standards of 1mg/L, 5mg/L, 10mg/L and 20mg/L were however prepared from the 100mg/L bulk phthalate standard for the quantification of the phthalates in the soxhlet extracts. The standards were stored in a fridge at a temperature of 5°C.

3.7 PREPARATION OF SPIKED SAMPLES

About 2.0g of polyethylene sample was accurately weighed in duplicate and one of the duplicate samples was spiked with known concentration (1ppm) of the prepared mixed phthalate standard in the extraction thimble. Both samples (spiked and unspiked) were taken through the extraction procedure under the same analytical conditions and analysed alongside the test materials using GC-MS. The concentrations of both the spiked and the unspiked samples were determined and the difference calculated as the concentration of the spiked. The efficiency of the analytical test method for migration was also validated by spiking a known weight (2.0g) of one of a duplicate sample with 5ppb of the prepared mixed phthalates standard. The samples were cut into 3x3cm pieces before weighing. Both the spiked and unspiked samples were taken through the migration analytical procedure described above. The concentrations of both the spiked and unspiked samples were determined and the difference represented the concentrations of the spiked samples. The percentage recoveries of the mean concentrations of the spiked samples were then calculated.

3.8 GC/MS ANALYSIS OF PHTHALATES IN POLYETHYLENE SAMPLES

The analysis of phthalates in polyethylene packaging materials using distilled water and olive oil as food simulants was conducted using a varian CP 3800 Gas Chromatography coupled with Saturn 2200 mass spectrometry. The GC was fitted with a programmable split/splitless injector; the injector-port temperature was maintained at 280°C. The injector line was packed with glass wool to improve vaporization and provide a surface for the collection of any dissolved plastic. A varian HP-5 (30m x 0.25mm x 0.25µm) GC column was temperature programmed from 100°C to 260°C at 8°C/min, then to 300°C at 35°C/min and held for 8.86 minutes. The carrier gas, helium was set at a constant flow of 1mL/min with 25ml/min split at the injector port. A string volume of 10µL was injected at a volume of 2µL at a normal speed. Ethyl acetate was used as the rinsing solvent.

A Saturn 2200 mass spectrometer equipped with a Varian CP8400 auto sampler as shown in **fig 3.2** was operated in full scan mode for this analysis. The scanning parameters were across a range of m/z 45-300. The heated zones were set at 80°C for the manifold, 210°C for the ion trap and 260°C for the transfer line to prevent condensation of the analytes.

Qualitative analysis of the phthalates was carried out by a Varian MS station software version 6.9

Fig.3.2 Varian CP 3800GC with Saturn 2200 MS

3.9 ANALYTICAL TEST METHODS EFFICIENCY

The extraction efficiency of the soxhlet extraction technique for the extraction of phthalates from the polyethylene packaging materials and the efficiency of the analytical test method for phthalate migration from the polyethylene packaging materials into both food simulants were determined as follows,

a) Known weights of about 2.0g of the polyethylene samples were soxhlet extracted for three times each with fresh ethyl acetate. The resultant extracts were analysed with GC-MS. The results indicated that the extraction was complete after the first extraction since no phthalate was detected in the subsequent extracts.

b) For the analytical test method for migration, known weights (2.0g) of polyethylene duplicate samples (spiked and unspiked) were analysed under the same analytical conditions and alongside the test materials. The difference in concentration between the spiked sample and the unspiked sample was calculated as the concentration of the spiked sample. The percentage recovery of the concentrations of the spiked samples were therefore determined and recorded.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.0 Results of Spiked Samples

The mean concentrations of the spiked samples with the percentage recovery are shown in **table 4.1**. The percentage recovery of the mean concentrations ranged from 67.10% to 125.20% for the 1ppm spiked samples and 88.34% to 116.98% for the 5ppb spiked samples.

Table.4.1. Results for spiked samples

MATRIX	1ppm spiked	5 ppb spiked	% recovery (1ppm)	% recovery (5ppb)
DEHP	0.888	5.298	88.80	105.96
DEP	0.671	4.417	67.10	88.34
BBP	1.252	5.849	125.20	116.98
DBP	1.170	5.526	117.00	110.52

4.1 QUALITATIVE IDENTIFICATION OF DEHP, DEP, BBP AND DBP IN POLYETHYLENE PACKAGES

To identify the presence of the four phthalates namely 2-di-(ethyl hexyl) phthalate(DEHP), di ethyl phthalate (DEP), Benzyl butyl phthalate (BBP) and Di-butyl phthalate (DBP) being analysed, the newly bought standards were prepared and then analysed with a GC-MS to confirm their identities. The retention times of the various phthalates were also determined and used to identify the compounds. The polyethylene samples of the five different categories were extracted and analysed with the GC-MS using the same method settings used to analyse the standards as described above in the analytical test method. The fragmentation patterns of the various samples being analysed were compared with those of the phthalate standards. All the four phthalates under investigation were detected and identified with the help of major fragmentation ions that corresponded with that of the gas chromatograms of the standards from the GC-MS and the identities of the phthalates confirmed using the GC-MS library data base. The major fragmentation ion detected in all the phthalates was represented by a mass to charge ratio of 149. This was found to be the protonated phthalic acid anhydride ion. Other additional ions such as 177.0 and 176.0 for DEP, 205.0 for DBP, 91.0 and 206.0 for BBP and 167.0 for DEHP were all identified hence were significant in the phthalates identification. Typical GC chromatograms and mass spectra of the mixed standard for the various phthalates, a chromatogram of a test sample and a blank sample are shown in **fig .4.1-.4.4**.

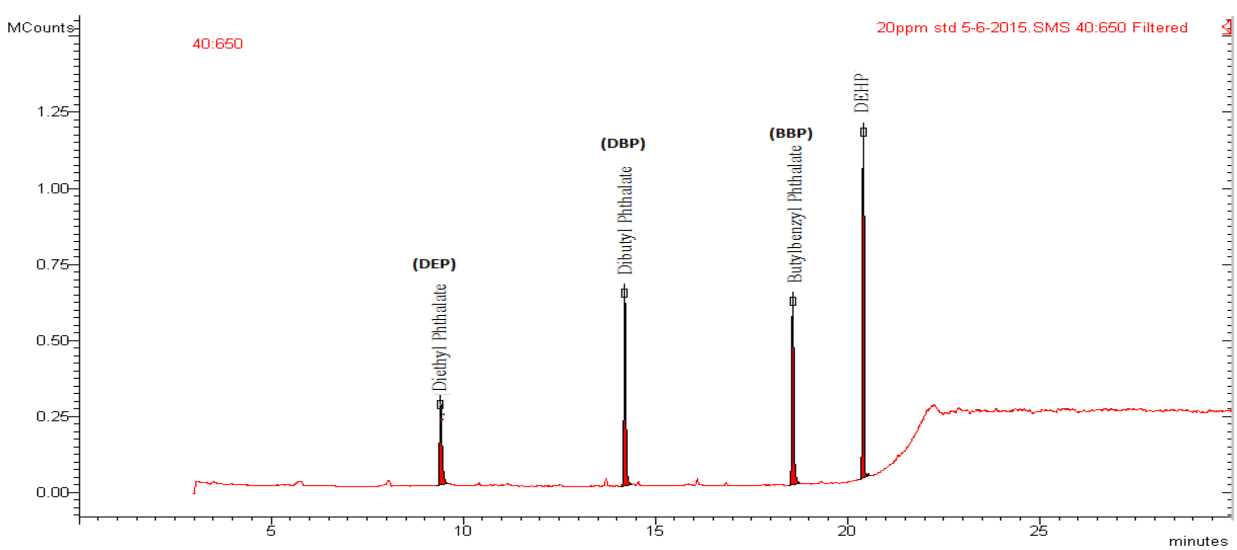


Fig.4.1. Gas Chromatogram of 20ppm mixed phthalates standard

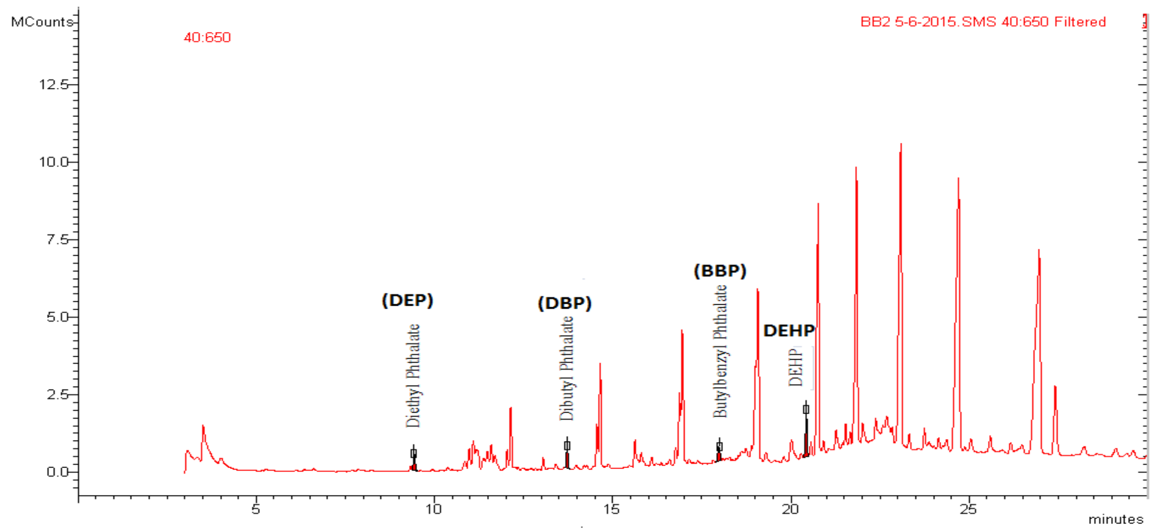


Fig.4.2. Gas Chromatogram of a Black Polyethylene Bag (BPB) sample

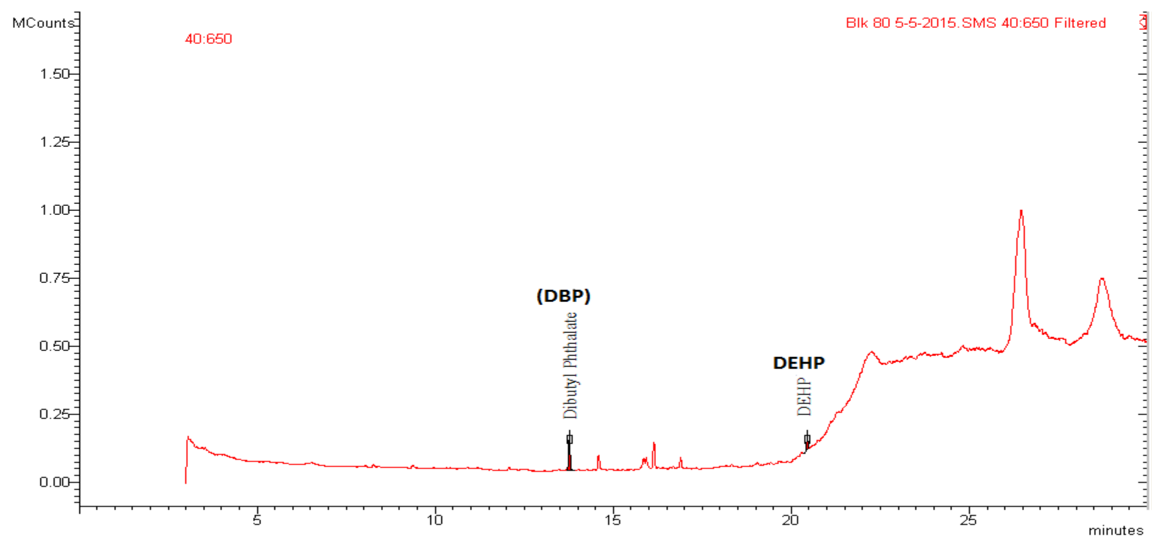


Fig. 4.3. Gas Chromatogram of a soxhlet extract blank sample.

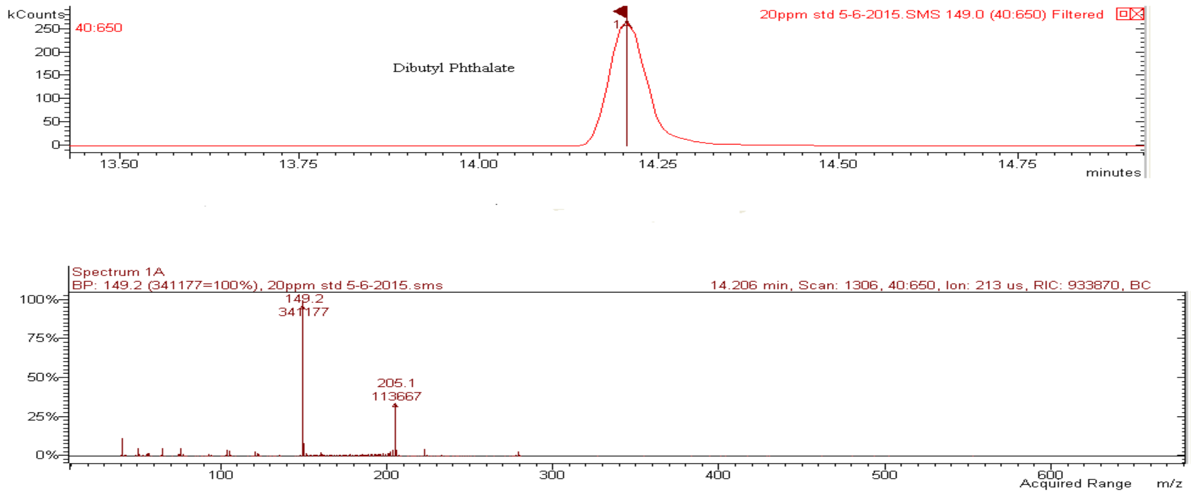


Fig.4.4a. Mass Spectrum of DBP

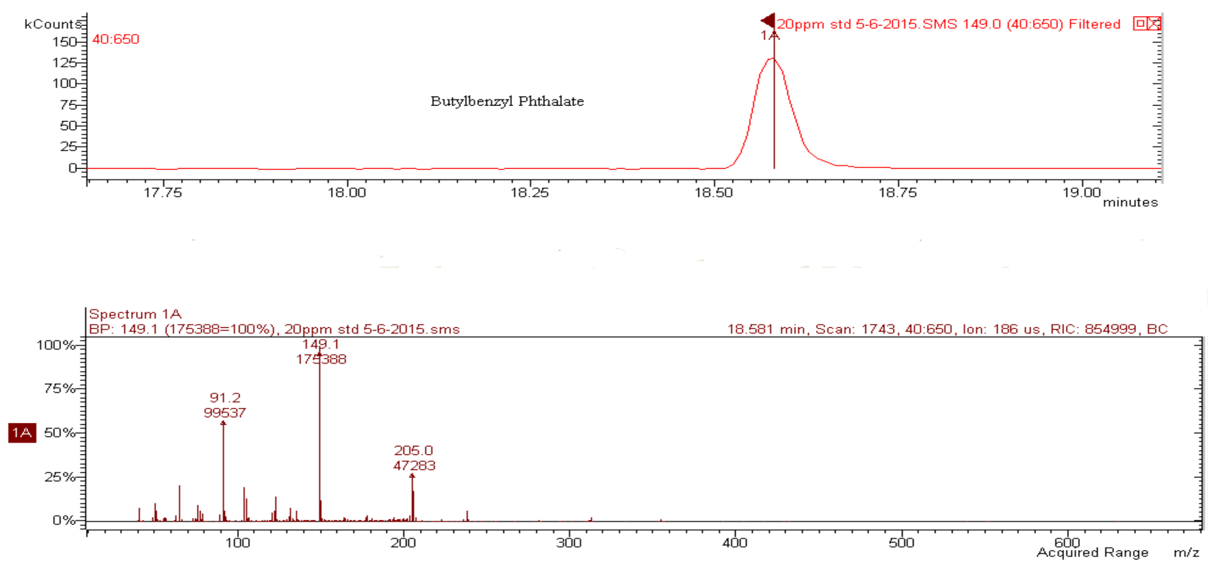


Fig. 4.4b. Mass Spectrum of BBP

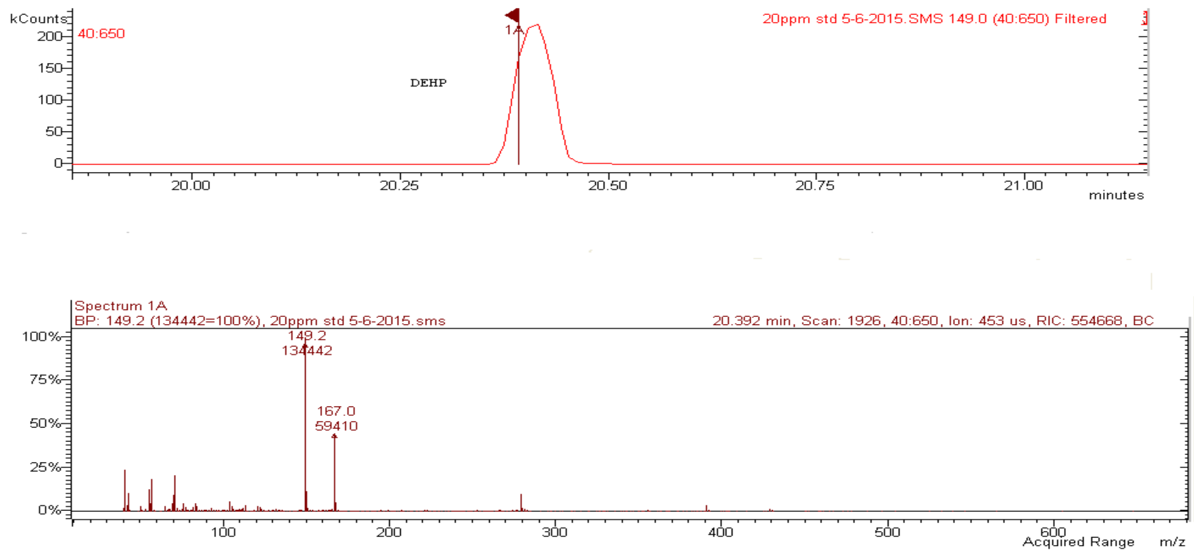


Fig.4.4c. Mass Spectrum of DEHP

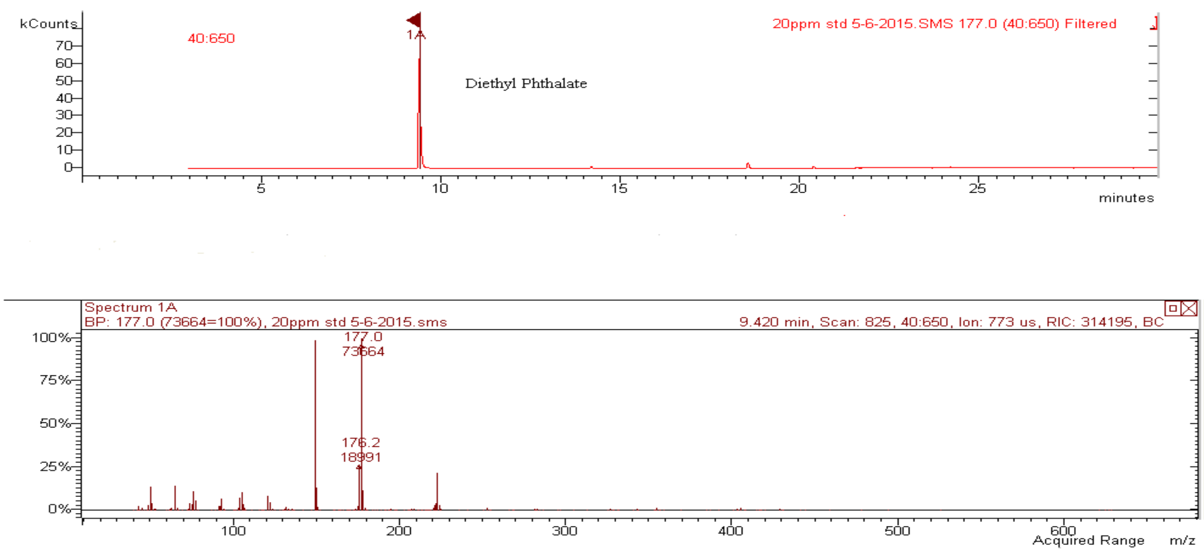


Fig.4.4d. Mass Spectrum of DEP

4.2 QUANTIFICATION OF DEHP, DEP, DBP AND BBP IN POLYETHYLENE PACKAGES

4.2.1 Determination of phthalates in black polyethylene bags (BPB)

Black Polyethylene Bags (BPB) of different brands sold on the Ghanaian market were purchased and analysed for the common four (4) phthalates found in foods and food packages namely, DEHP, DEP, BBP and DBP. Results are shown in **table 4.2.** All the phthalates were detected in all the black polyethylene bags with the exception of BBP which was detected in only one of the five black polyethylene packaging samples analysed. Di 2-(ethyl hexyl) Phthalates (DEHP) was detected in significant quantities ranging from 9.89mg/kg to 14.30mg/kg with an average value of 12.03mg/kg in all the BPB samples. DEHP, which is the most common and widely detected phthalate in food and food packages account for about 50% of the world production of phthalates as reported by Wenzl Thomas in an outcome of a survey conducted among European food control laboratories [25]. DEP was detected in trace quantities in most of the samples. DBP was also dominant in all samples but at lower levels compared to DEHP as shown in **fig 4.5.**

Table 4.2 Mean Concentrations of phthalates in black polyethylene bags.

Phthalates	Mean conc. (mg/kg)	Standard deviation	Minimum(mg/kg)	Maximum (mg/kg)
DEHP	12.03	2.11	9.89	14.30
DEP	2.19	1.67	0.75	4.97
DBP	8.03	3.80	3.59	12.35
BBP	0.29	0.63	ND	1.43

ND= Not detected

From **table 4.2**, the values detected for DEP and DBP ranged from 0.75mg/kg to 4.97mg/kg and 3.59mg/kg to 12.35mg/kg with average values of 2.19mg/kg and 8.03mg/kg respectively. BBP was detected in only one out of the five different brands of black polyethylene bags analysed with a value of 1.43mg/kg. A study by Moreira et al. on the migration of phthalates to food simulants in plastic containers during microwave heating also reported similar results where BBP was not detected in any of the samples analysed [6]. In addition, Shen in a study according to Moreira et al. also reported 0.10mg/kg as the average concentration of BBP detected in plastic containers used in microwave ovens [6]. This is an indication that BBP are detected in trace amounts in plastic food packaging materials. A study on the migration of phthalates in printed polyethylene confectionary packages by Balafas et al. also reported DEHP and DBP as the predominant phthalates detected in all the samples [9].

Fig 4.5 compares the mean levels of phthalates detected in the BPB samples analysed.

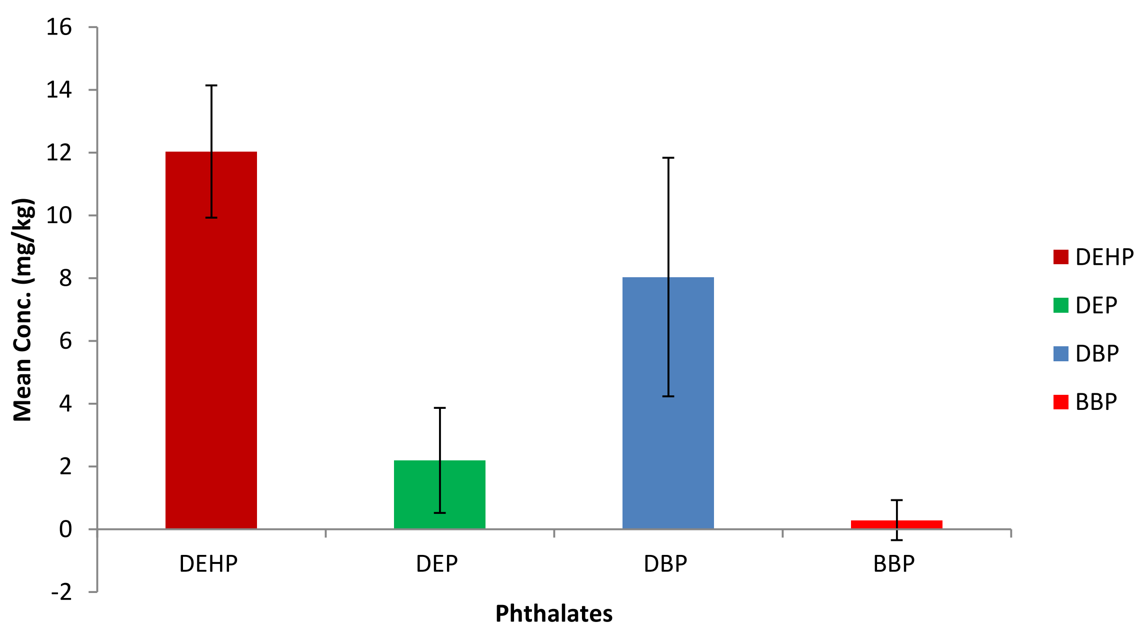


Fig.4.5. Comparison of Phthalate concentrations found in Black Polyethylene Bag (BPB)

4.2.2 Determination of phthalates in take away bags (TAB)

Five different brands of Polyethylene films/bags sold on the Ghanaian market and popularly known as Take Away Bags (TAB) were purchased and analysed. Take away bags is one of the most common polyethylene packaging materials used in the Ghanaian food industry in which most local foods such as waakye, red-red, fufu with soup, banku, kenkey are packaged at the point of sale.

Table.4.3. Mean concentrations of phthalates in TAB

Phthalates	Mean conc. (mg/kg)	Standard deviation	Minimum(mg/kg)	Maximum (mg/kg)
DEHP	3.08	3.31	ND	7.99
DEP	2.47	2.61	ND	5.32
DBP	11.35	2.82	8.87	15.45
BBP	ND	ND	ND	ND

As shown in **table 4.3**, BBP was not detected in any of the five samples of the take away bags analysed. DEHP and DBP were however detected in significant quantities ranging from not detected to 7.99mg/kg and from 8.87mg/kg to 15.45mg/kg respectively with average values of 3.08mg/kg and 11.35mg/kg. DEP was also detected in significant quantities ranging from not detected to 5.32mg/kg with a mean value of 2.47mg/kg. The predominant phthalates in the take away bags were DBP followed by DEHP and DEP. **Fig 4.6** below compares the various phthalates concentrations detected in the TAB

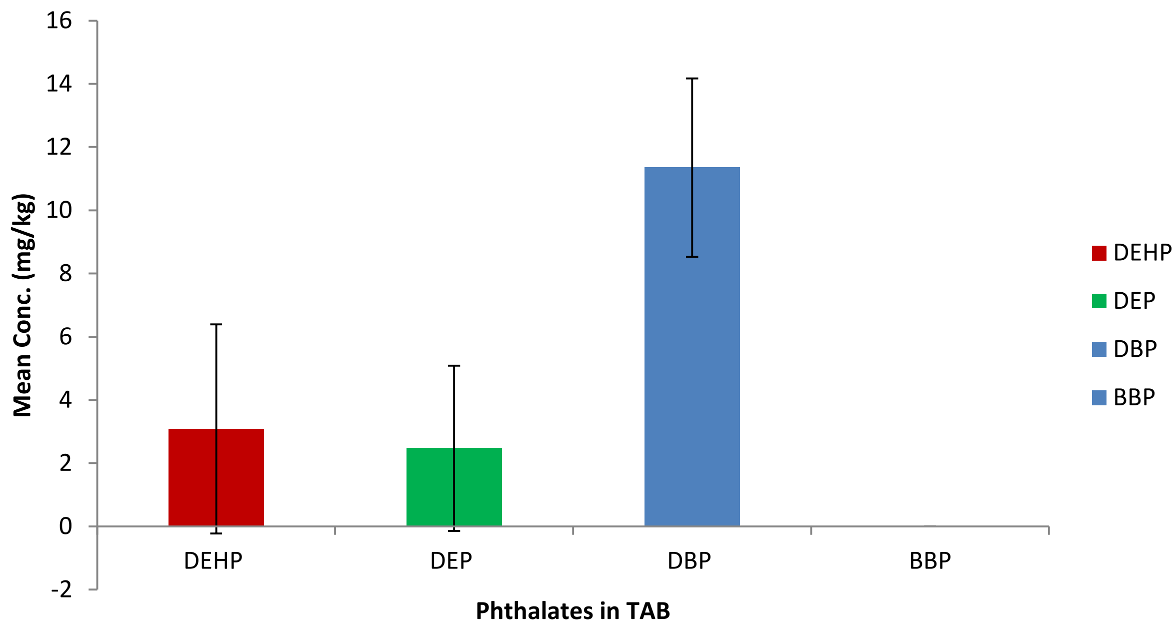


Fig.4.6. Comparison of Phthalates concentrations found in Take Away Bags

4.2.3 Determination of phthalates in Thick Polyethylene Bags (TPB)

Thick polyethylene plastic bags (TPB) or films are mostly used in the food industry and sold on the Ghanaian market. These polyethylene films/bags are either in the form of HDPE or LDPE and are all used for the same purpose. These plastic packages are used in the food industry at very high temperatures. They are mostly used to cover local foods such as kenkey, banku and sometimes rice during cooking. **Table 4.4** and **fig 4.7** below shows the mean concentrations of the various phthalates detected in TPB.

Table.4.4. Mean concentrations of phthalates in TPB

Phthalates	Mean conc. (mg/kg)	Standard deviation	Minimum(mg/kg)	Maximum (mg/kg)
DEHP	5.84	4.18	ND	10.73
DEP	1.68	1.88	ND	4.10
DBP	9.94	2.93	5.71	13.86
BBP	ND	ND	ND	ND

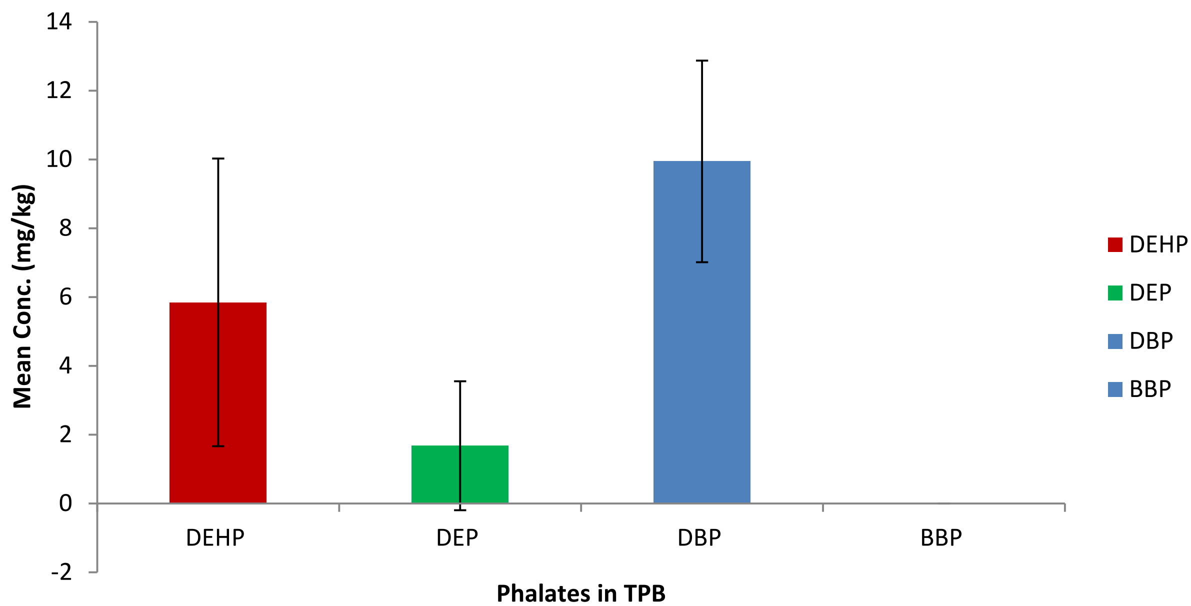


Fig.4.7. Comparison of Phthalates concentrations found in Thick Polyethylene Bags (TPB)

The five different brands of the TPB samples analysed showed high concentrations of DBP ranging from 5.71mg/kg to 13.86mg/kg with a mean value of 9.94mg/kg. BBP however was not detected in any of the five samples analysed. DEHP and DEP showed significant values ranging from not detected to 10.73mg/kg and not detected to 4.10mg/kg with average values of 5.84mg/kg and 1.68mg/kg respectively. The trend of phthalates detected in the thick polyethylene plastic bags was similar to that detected in the take away polyethylene bags where DBP was predominant followed by DEHP and DEP. Various research works on food and food packaging materials (Shen [6], Miriany et al. [6], and Bonini et al. [24]) have all reported similar results where the predominant phthalate is either DEHP or DBP.

4.2.4 Determination of phthalates in polyethylene plastic bottles (PPB)

Polyethylene Plastic Bottles (PPB) is one of the commonly used plastic products in the beverage industry. As shown in **table 4.5** below, DEP was detected in trace concentrations in all the PPB samples ranging from 0.37mg/kg to 1.31mg/kg with an average value of 0.84mg/kg. Benzyl Butyl Phthalate (BBP) was not detected in any of the plastic bottles. DBP and DEHP levels detected corresponded to the outcome of the survey conducted by the European food control laboratories in 2009 [25]. The concentration values ranging from 5.11mg/kg to 9.66mg/kg and not detected to 10.60mg/kg respectively for DBP and DEHP. The mean values for DBP and DEHP detected in all the plastic bottles were 7.43mg/kg and 4.94mg/kg respectively. The concentrations of these phthalates are represented graphically in **fig.4.8**

Table.4.5. Mean concentrations of phthalates in polyethylene plastic bottles

Phthalates	Mean conc. (mg/kg)	Standard deviation	Minimum(mg/kg)	Maximum (mg/kg)
DEHP	4.94	4.10	ND	10.60
DEP	0.84	0.34	0.37	1.31
DBP	7.43	1.94	5.11	9.66
BBP	ND	ND	ND	ND

ND=Not Detected

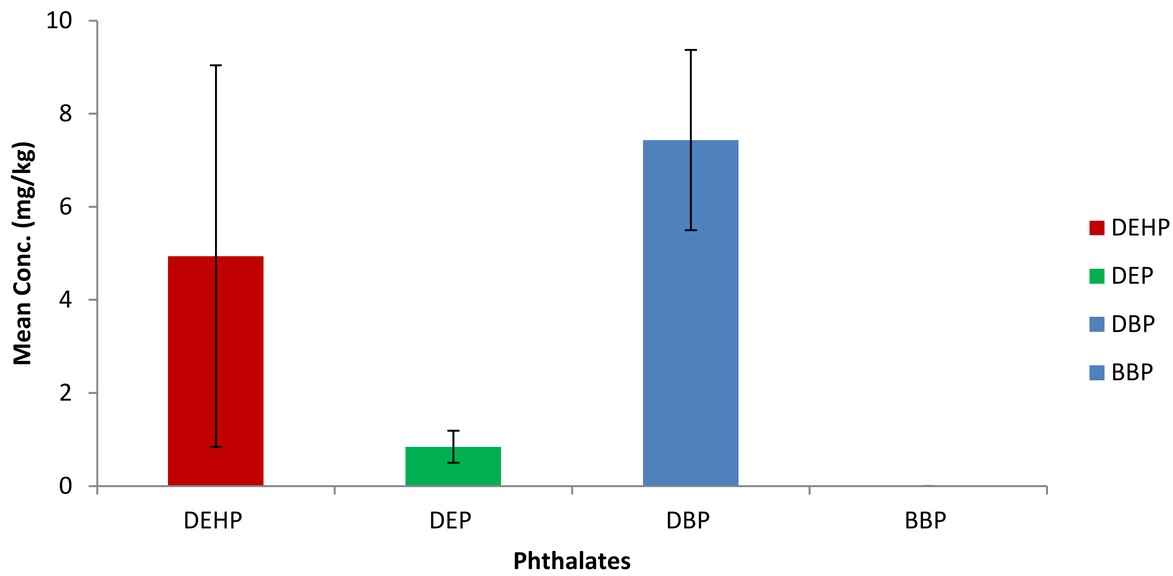


Fig.4.8. Comparison of Phthalates concentrations found in Polyethylene Plastic Bags (PPB)

4.2.5 Determination of phthalates in polyethylene food containers (PFC)

Polyethylene Food containers (PFC) which make up the final category of the polyethylene food packages under investigation mostly come in the form of High Density Polyethylene (HDPE). The mean concentrations of the various phthalates detected in these food containers are shown in **table 4.6**.

Table.4.6. Mean concentrations of phthalates in polyethylene food containers

Phthalates	Mean conc. (mg/kg)	Standard deviation	Minimum(mg/kg)	Maximum (mg/kg)
DEHP	7.35	4.48	ND	12.08
DEP	1.26	0.28	1.05	1.73
DBP	7.95	2.76	4.92	11.78
BBP	ND	ND	ND	ND

Fig 4.9 compares the concentrations of the various phthalates detected in the food containers.

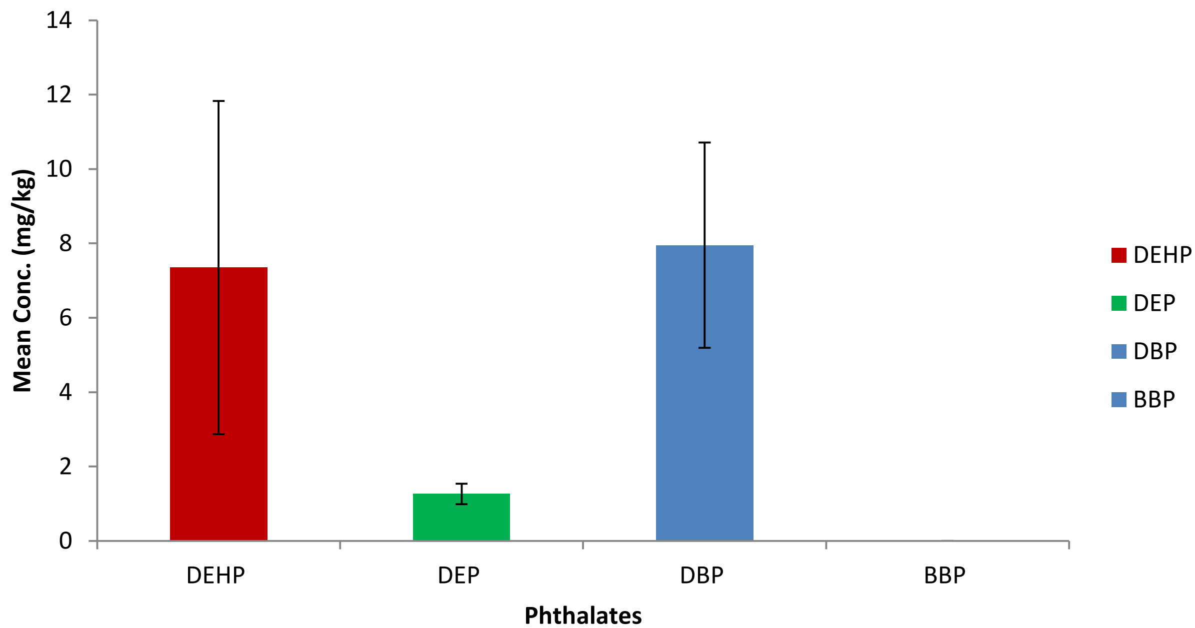


Fig.4.9. Comparison of Phthalates concentrations found in Polyethylene Food Containers

The mean values for DBP, DEHP and DEP detected in the plastic food containers as shown in **table 4.6** were 7.95mg/kg, 7.35mg/kg and 1.26mg/kg respectively. DBP was detected in all the food containers ranging from 4.92mg/kg to 11.78mg/kg followed by DEHP. DEHP concentrations ranged from not detected to 12.08mg/kg. DEP was detected in all samples but in lower concentrations ranging from 1.05mg/kg to 1.73mg/kg. BBP however was not detected.

4.2.6 Average phthalates concentrations determined in the polyethylene packages

The **table 4.7** shows the average phthalates concentrations detected in each of the five categories of polyethylene plastic packaging materials**.** As observed in **table 4.7**, BBP was detected in only one sample out of the 25 polyethylene food packages analysed with a value of 1.43mg/kg. DEHP which was one of the predominant phthalates was detected in 20 samples ranging from not detected to 14.30mg/kg with a mean value of 6.65mg/kg. DEHP was dominant in the black polyethylene bags with a mean value of 12.03mg/kg. DEHP however recorded the lowest mean value of 3.08mg/kg in the take away polyethylene bags.

Table.4.7. Average concentrations and frequency of occurrence of phthalates in polyethylene packaging materials

Phthalates	Mean conc. (mg/kg)	Minimum (mg/kg)	Maximum (mg/kg)	Frequency of occurrence Out of 25 samples
DEHP	6.65±4.60	ND	14.30	20
DEP	1.69±1.61	ND	5.32	21
DBP	8.94±3.06	3.59	15.45	25
BBP	0.06±0.27	ND	1.43	1

Shen found similar concentrations of 8.67mg/kg for DEHP in plastic food containers used for microwave ovens [6]. Shen in addition reported an average concentration value of 1.44mg/kg for DBP in polypropylene food containers which are however lower than what is reported in this research. A research report by Bhunia et al. mentioned DEHP, DEP, DBP and BBP among other chemical migrants as the common plasticizers found in polyethylene packaging materials [28]. These phthalates in addition to other additive are used to enhance the performance of polymers during processing and fabrication per the report.

From **table 4.7**, di-butyl phthalate (DBP) was detected in all the 25 samples analysed with a significant mean value of 8.94mg/kg ranging from 3.59mg/kg to 15.45mg/kg with the lowest mean value of 7.43mg/kg detected in the polyethylene plastic bottles and the highest mean concentration of 11.35mg/kg detected in the take away polyethylene bags.

Di-Ethyl Phthalate (DEP), one of the phthalates that was detected in trace quantities was detected in 21 out of the 25 samples with a mean value of 1.69mg/kg ranging from not detected to 5.14mg/kg. The highest mean concentration of 2.47mg/kg was detected in the take away polyethylene bags with the lowest mean value of 0.84mg/kg detected in the polyethylene plastic bottles.

Wittasek et al. assessed human exposure to phthalates and reported that long chain phthalates such as DEHP are mostly found in food packages and other products such as building and construction materials whilst the short chain phthalates such as DEP and BBP are used in applications such as personal care products and enteric-coated tablets [30]. This may explain the reason why DEP and BBP were not detected in some of the samples or detected in trace concentrations compared to the concentrations of DEHP and DBP.

Heise and Litz also confirmed that only two of the phthalates are regularly found in environmental samples, primary DEHP and to a much lesser degree DBP [19].

A graphical representation shown in **fig 4.10** compares the average concentrations of the phthalates detected in the polyethylene packaging materials;

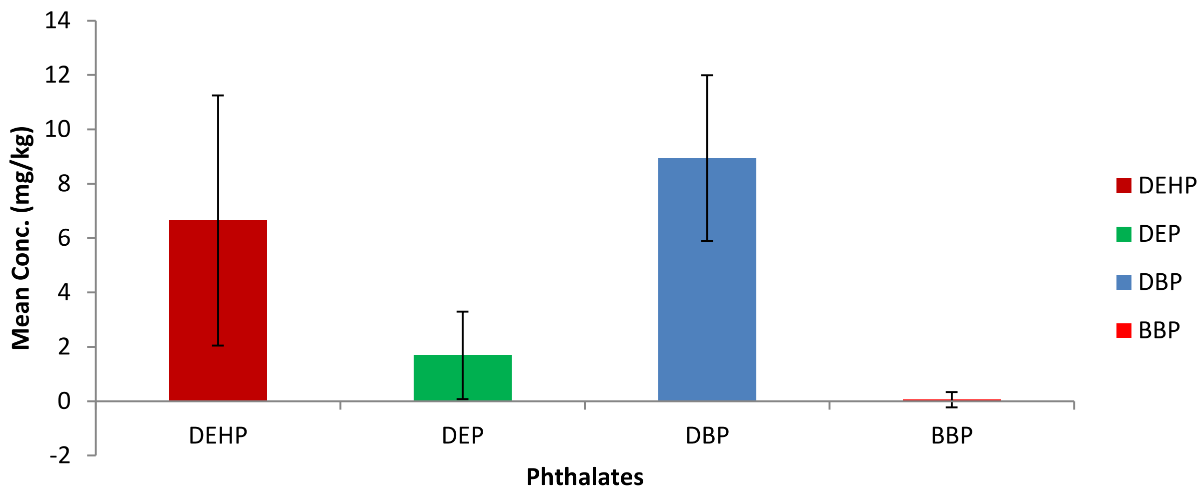


Fig.4.10. Comparison of phthalate concentrations found in polyethylene packaging materials

4.3 MIGRATION OF PHTHALATES FROM POLYETHYLENE PACKAGES INTO DISTILLED WATER

The migration of substances from a packaging material to food during storage or heating depends on several factors such as the temperature of contact, the duration of contact, nature of the migratory substance, surface area of contact and the nature of the food. Hence to evaluate the effect of temperature and the contact time on the migration of phthalates namely; DEHP, DBP, DEP and BBP from polyethylene packaging materials into food using distilled water as food simulant involved three (3) steps

1) The first step was to expose the polyethylene packaging materials to the food simulant at different temperature and different contact times and then allow the substance from the packaging material to migrate into the food simulant.

2) The second step was to transfer the migrants from the food simulant (aqueous medium) to an ethyl acetate (organic medium) by extraction using a separatory funnel.
3) The third and final step involved the quantification of the migrants transferred from the packaging material to the food simulant in terms of specific migration using a GC-MS.

The migration test was performed according to the indication of the European commission regulation No. 10/2011 in which distilled water is used as a food simulant for aqueous foods. Polyethylene samples that showed a detectable content of phthalates after the extraction assays were exposed to the food simulant at different contact times and different temperatures. The packaging materials were cut into pieces of equal sizes to ensure an equal contact surface area of the material with the food simulant. The migrated amounts were determined by the same GC-MS procedure applied for the soxhlet extracts. The migrated concentrations of the phthalates were however calculated in µg/kg due to the minute quantities detected after the various times exposure at the different temperatures. Each sample was analysed in duplicates. DEHP and DBP were detected in all samples. BBP was not detected in any of the samples analyst whilst DEP was detected in trace amounts only at high temperatures during longer time exposures. DEP is used as a plasticizer in various cosmetic and personal care products and other cosmetic ingredients including medical treatment tubing [19].

4.3.1 PHTHALATES MIGRATION IN POLYETHYLENE PACKAGES AT CONSTANT TEMPERATURE WITH VARYING EXPOSURE TIMES

4.3.1.1 Migration of DEHP from the plastics at constant temperatures

The mean concentrations of DEHP that migrated from the various polyethylene packages at different times at a constant temperature of 5°C is shown graphically in **fig. 4.11a**.

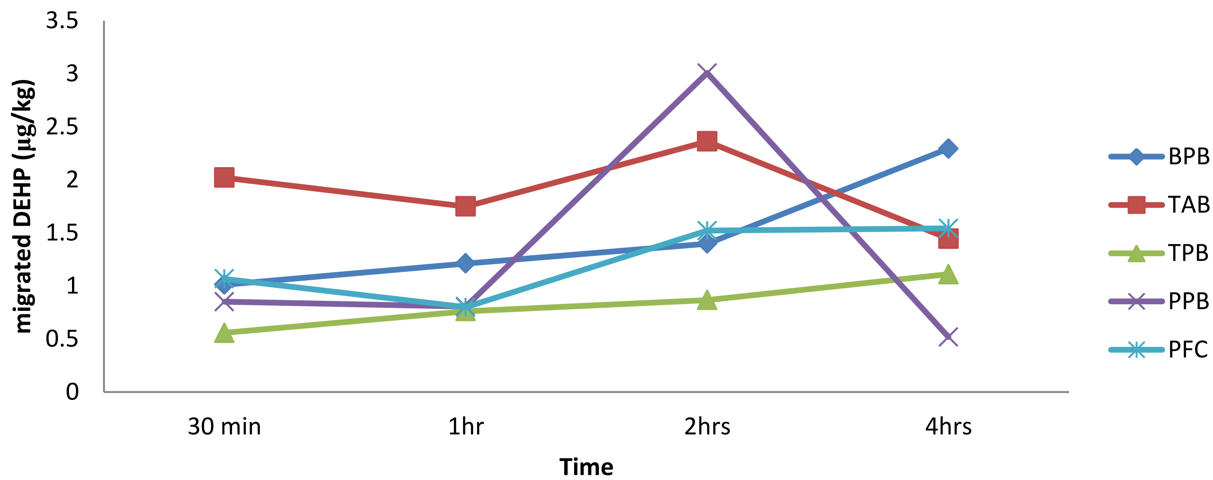


Fig.4.11a. Concentrations of migrated DEHP from the plastic materials at 5°C

Table.4.8. Mean concentrations of migrated DEHP at 5°C in distilled water

Time	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
30mins	1.01±0.11	2.02±0.09	0.56±0.18	0.85±0.12	1.07±0.11
1hr	1.21±0.10	1.75±0.22	0.76±0.10	0.81±0.54	0.80±0.04
2hrs	1.40±0.02	2.36±0.18	0.87±0.07	3.01±0.04	1.52±0.07
4hrs	2.30±0.04	1.45±0.10	1.11±0.13	0.52±0.01	1.54±0.10

The concentrations of DEHP that migrated from the packaging materials varied randomly from one packaging material to another with the lowest concentration recorded by the TPB after the 30minutes contact time at 5°C and the highest concentration detected in the PPB at the 2 hours contact time as observed in **table 4.8**. The average concentrations of DEHP that migrated from BPB, TAB, TPB, PPB and PFC were 1.01µg/kg, 2.02µg/kg, 0.56µg/kg, 0.85µg/kg and 1.07µg/kg respectively after the 30minutes contact time at 5°C. The migrated concentrations of DEHP after the 4hours exposure time at 5°C however recorded the following values of 2.29μg/kg, 1.45μg/kg, 1.11μg/kg, 0.52μg/kg and 1.54μg/kg for BPB, TAB, TPB, PPB and PFC respectively indicating that there was no any linear correlation between the migrated concentrations of DEHP and the varied times of contact though phthalate migration was observed as indicated in **fig.4.11a**.

DEHP migration was detected in all the samples in trace quantities ranging from 0.20μg/kg for PPB to 3.59μg/kg for BPB. A similar study conducted by Moreira et al.[6] on plastic containers used in microwave oven also reported the concentration of DBP from <LOD to 2.0μg/L and <LOD to 7.5μg/L for new and used containers respectively which are not much different in concentration from that reported in this work.

A research study, toxicity and exposure assessment for children's health, phthalates reported by U.S.A EPA stated that DEHP when absorbed into the body increases liver weight which affects its functions such as impairing bile excretion by the liver. The report also mentioned the effects of DEHP to include causing testicular atrophy and infertility in male organs when accumulated in male human body [29].

The phenomenon in which the concentrations of the migrated DEHP are random and do not show any linear correlation between the migrated DEHP concentrations and the contact times was repeated in all the analysis of DEHP migration at constant temperatures with varied exposure time conditions as shown in **fig. 4.11b-c.**

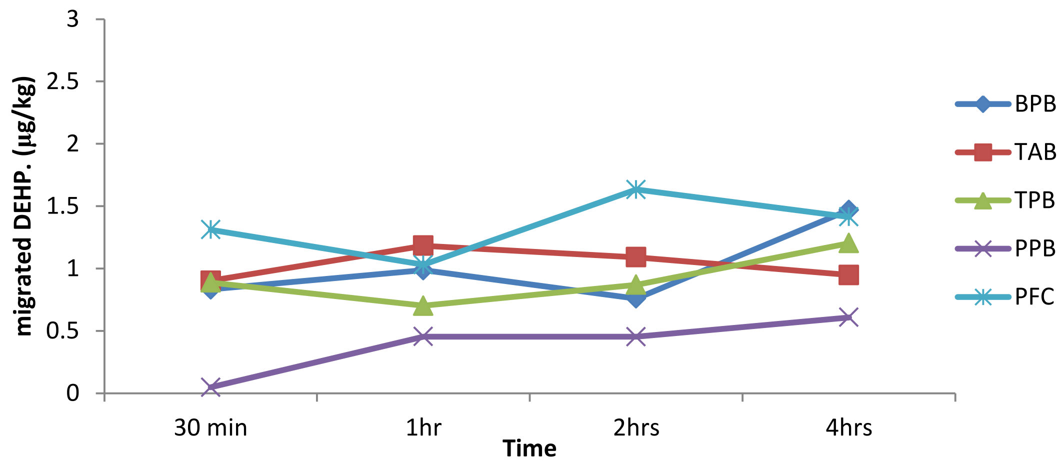


Fig.4.11b. **Concentrations of migrated DEHP from the plastic materials at 20°C**

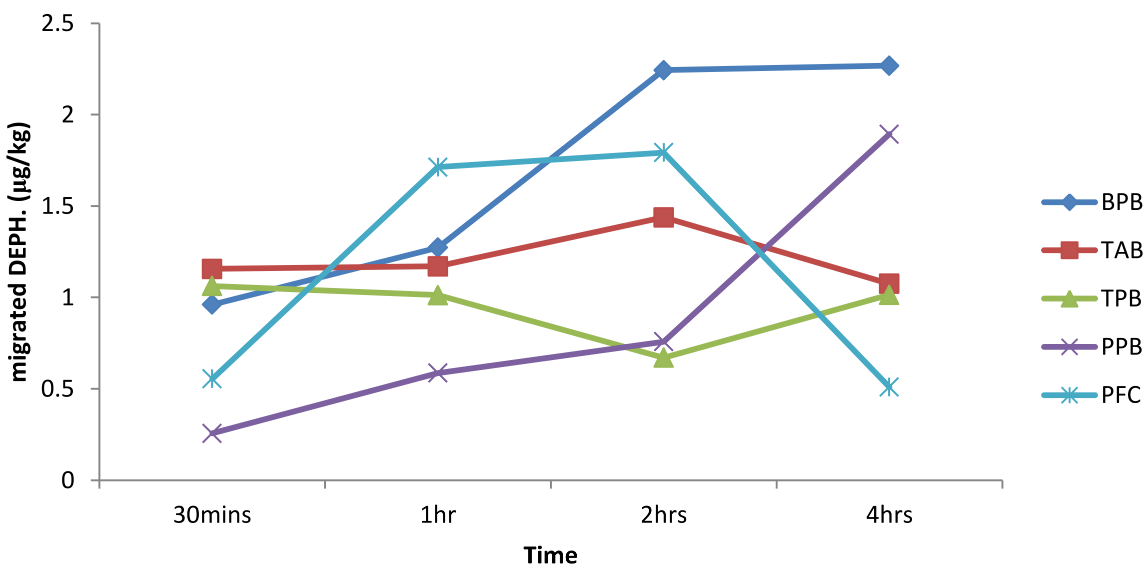


Fig.4.11c. Concentrations of migrated DEHP from the plastic materials at 40°C

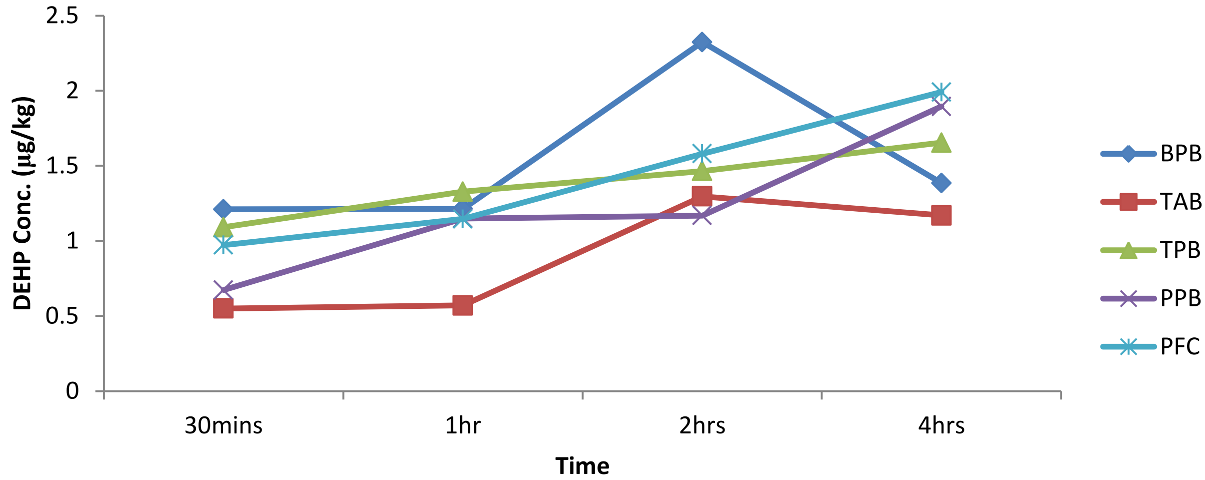


Fig.4.12a. Concentrations of migrated DEHP from the plastic materials at 80°C

Table.4.9. Mean concentrations of migrated DEHP at 80°C

Time	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
30mins	1.21±0.06	0.55±0.06	1.09±0.16	0.67±0.10	0.97±0.07
1hr	1.21±0.10	0.57±0.03	1.33±0.38	1.15±0.03	1.15±0.03
2hrs	2.32±0.15	1.30±0.04	1.47±0.36	1.17±0.03	1.58±0.53
4hrs	1.39±0.13	1.17±0.06	1.65±0.09	1.90±0.12	2.00±0.12

4.3.1.2 Migration of DBP from the plastics at constant temperatures

From **fig 4.12a** and **fig.4.12b**, the polyethylene packaging materials were exposed to the food simulant (distilled water) at a temperature of 80°C for four (4) hours. Thus the simulant remained in contact with the packaging materials at a constant temperature for a longer time which resulted in a different trend from that observed at the constant lower temperatures and the short time exposures as seen in **fig 4.11a-c.**

Table.4.10. Mean concentration of migrated DBP at 80°C in distilled water

Time	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
30mins	0.03±0.01	0.05±0.02	0.02±0.01	0.05±0.04	0.05±0.09
1hr	0.08±0.04	0.07±0.02	0.16±0.02	0.18±0.01	0.18±0.05
2hrs	1.08±0.11	0.07±0.01	0.14±0.01	1.11±0.17	0.29±0.02
4hrs	1.16±0.35	0.91±0.08	2.00±0.01	1.05±0.54	0.49±0.11

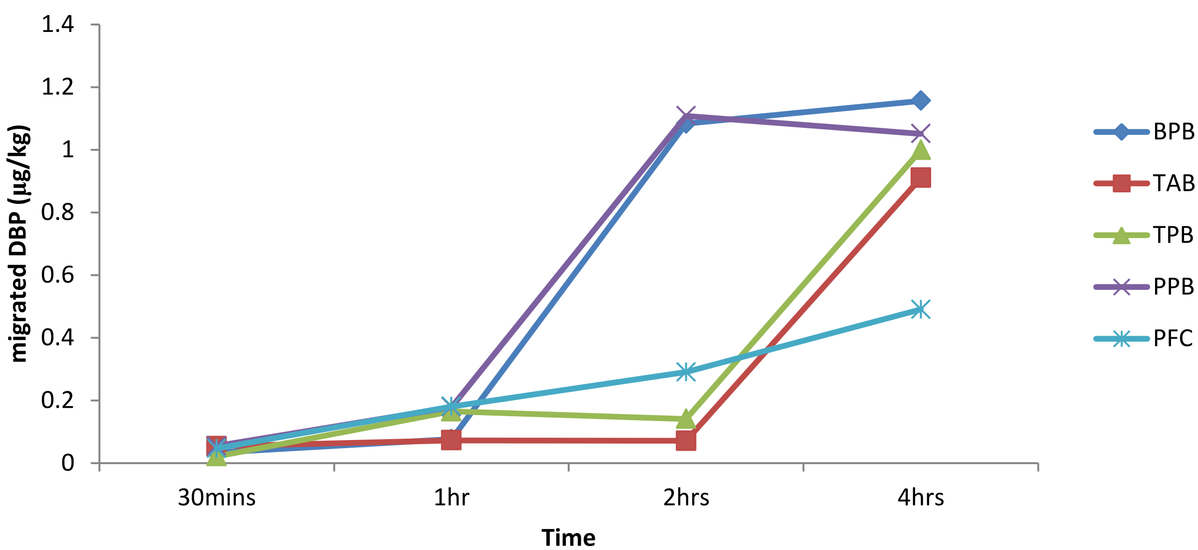


Fig.4.12b. Concentrations of migrated DBP from the plastic materials at 80°C

The concentrations of the migrated phthalates (DEHP and DBP) increased gradually in concentration from the 30minutes contact time to the 4 hours contact time at the 80°C. Thus the longer exposure time of the packaging materials with the food simulant at a higher temperature might have resulted in the linear correlated increased of the phthalates migration to the food simulant. A research by Bonini et al. reported that the migration of phthalates from plastic containers do not depend on the time of contact or length of exposure [24]. The results of this work can however corroborate this fact only at lower temperatures. But at higher temperatures and longer exposure times, the concentrations of the phthalates migrated into the food simulant definitely increased. For instance, in **fig 4.12a**, the migration of DEHP increased gradually in concentration from 0.55µg/kg for TAB, 0.97µg/kg for PFC and 0.67µg/kg for PPB at the 30 minutes contact time to 1.17µg/kg, 1.99µg/kg and 1.90µg/kg at the 4 hour contact time respectively.

Fig 4.13a-c below shows the DBP migration at lower temperatures with varied time conditions for 30 minutes, 1hour, 2hours and 4hours at constant temperatures of 5°C 20°C and 40°C. The concentrations of the migrated DBP are random hence an indication that there was no any linear correlation between the concentrations of the DBP migrated and the contact times or exposure times of the packaging materials at those temperatures.

Di benzyl Phthalate (DBP), one of the common phthalates mostly found in food packaging material with a molecular weight of 278.34 have serious adverse effects on humans when consumed in greater quantities. For instance, Soharu opined that DBP causes infertility in male organism by interfering with the male reproductive tract development when accumulated in the system. This is because they are toxic to cells in the testes that are responsible for normal sperm and hormone reproduction [7].

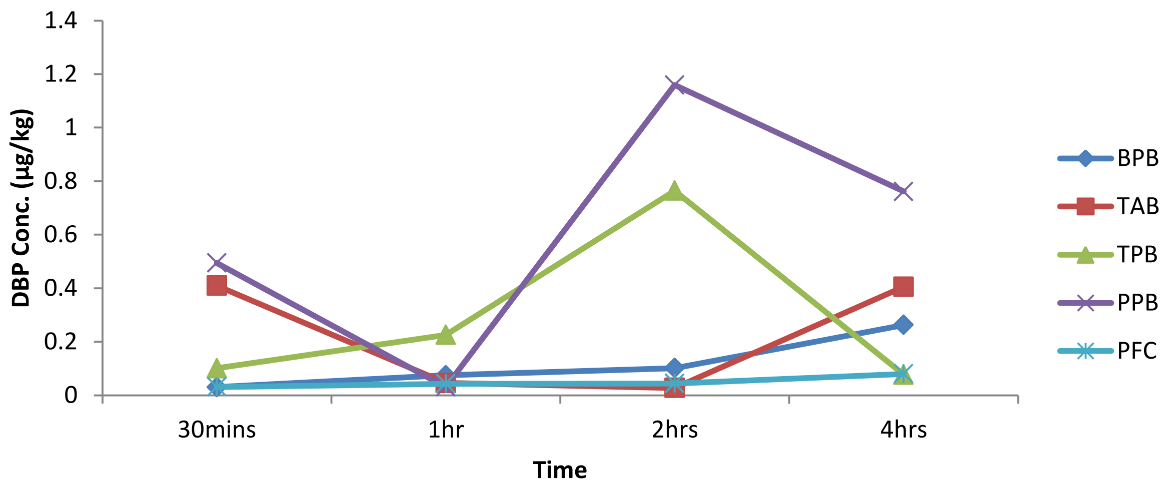


Fig.4.13a. Concentrations of migrated DBP from the plastic materials at 5°C

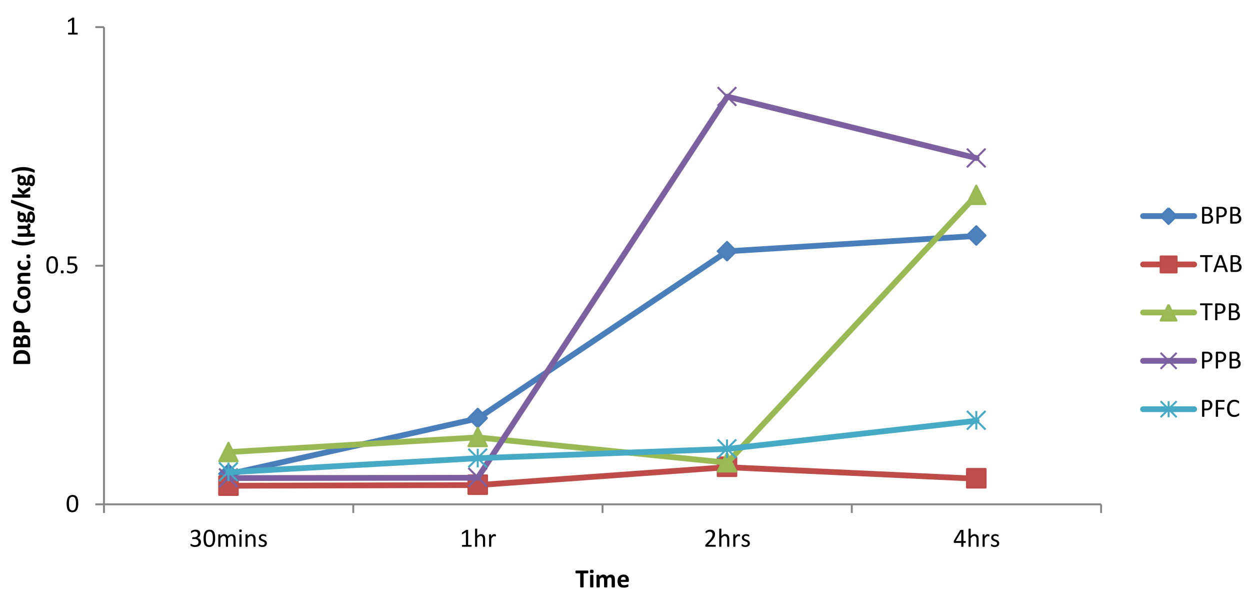


Fig.4.13b. Concentrations of migrated DBP from the plastic materials at 20°C

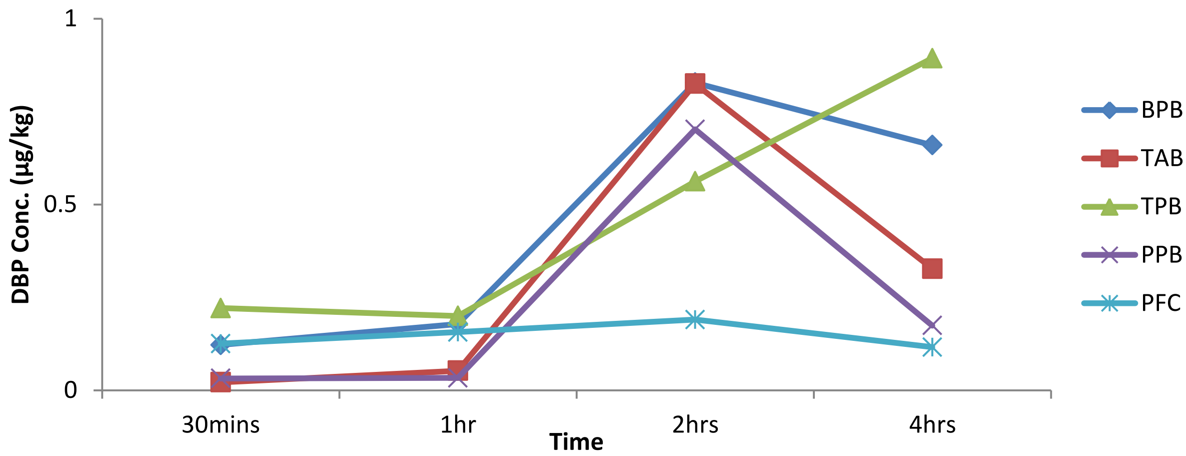


Fig.4.13c. Concentration of migrated DBP from the plastic materials at 40°C

4.3.2 PHTHALATES MIGRATION IN POLYETHYLENE PACKAGES AT CONSTANT TIME WITH VARYING TEMPERATURES

4.3.2.1 Migration of DEHP from the plastics at constant exposure times

The migration of phthalates from polyethylene packaging materials to food using distilled water as food simulant was also analysed at constant exposure times or contact times with varying temperatures. The packaging materials were exposed to the food simulant at constant exposure times of 30 minutes, 1 hour, 2 hours and 4 hours at varying temperatures of 5°C, 20°C, 40°C and 80°C. The concentrations of DEHP migrated at the 30minute and 1 hour exposure times are shown below in **table 4.11** and **table 4.12.**

The concentration of the migrated phthalates increased with increased in temperature as observed in **figs 4.14a-b.** The 30 minutes exposure time for DEHP as indicated below shows a continuous increase in the migrated DEHP concentration from the 5°C to the 80°C in all the packaging materials. For example the migrated concentration of DEHP for the black polyethylene bags increased from 0.83µg/kg at the 5°C continuously to a concentration of 1.21µg/kg at the highest temperature of 80°C. These trend of continuous increased in the concentration of the migrated DEHP as temperature increases was observed in all the migration analysis of phthalates in the polyethylene packaging materials. These are graphically represented in **figs 4.14a-d**

Table4.11. Mean concentrations of migrated DEHP for 30 mins in distilled water

Temperature(°C)	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
5	0.83±0.2	0.30±0.09	0.56±0.18	0.26±0.08	0.56±0.08
20	0.96±0.10	0.40±0.04	0.89±0.20	0.43±0.04	1.07±0.08
40	1.01±0.11	1.07±0.01	1.06±0.08	0.85±0.12	1.31±0.05
80	1.21±0.06	1.25±0.06	1.09±0.16	0.97±0.07	1.67±0.10

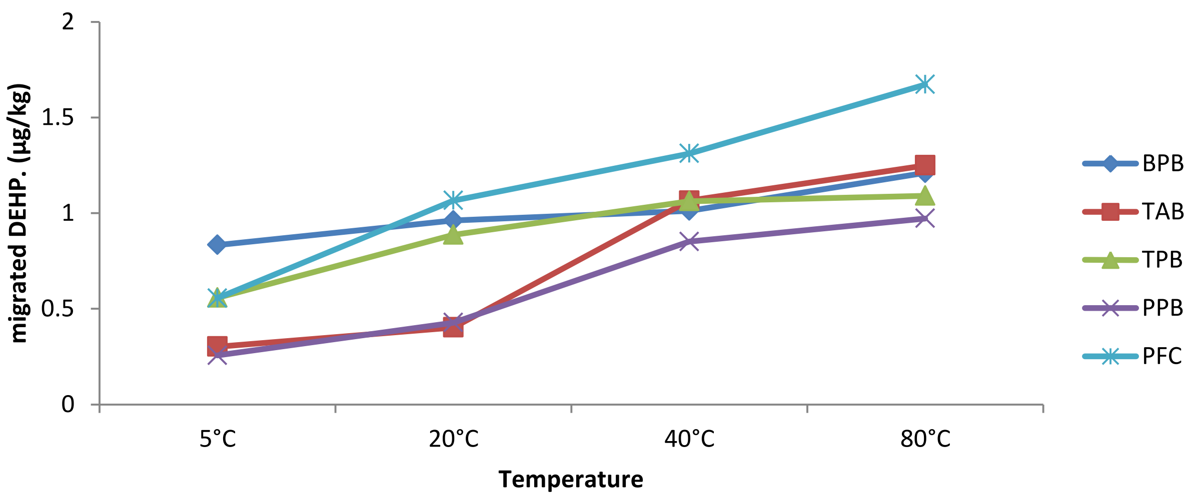


Fig.4.14a. Concentrations of migrated DEHP from the plastic materials for 30mins

Table 4.12. Mean concentrations of migrated DEHP for 1hr in distilled water

Temperature(°C)	BPB(μg/kg)	TAB(μg/kg)	TPB(μg/kg)	PPB(μg/kg)	PFC(μg/kg)
5	1.21±0.10	0.57±0.03	0.76±0.10	0.39±0.02	0.80±0.04
20	1.21±0.10	0.75±0.05	0.70±0.08	0.45±0.05	1.03±0.08
40	1.27±0.21	1.17±0.01	1.01±0.27	0.81±0.01	1.71±0.55
80	1.99±0.18	1.18±0.05	1.33±0.38	1.15±0.03	1.80±0.38

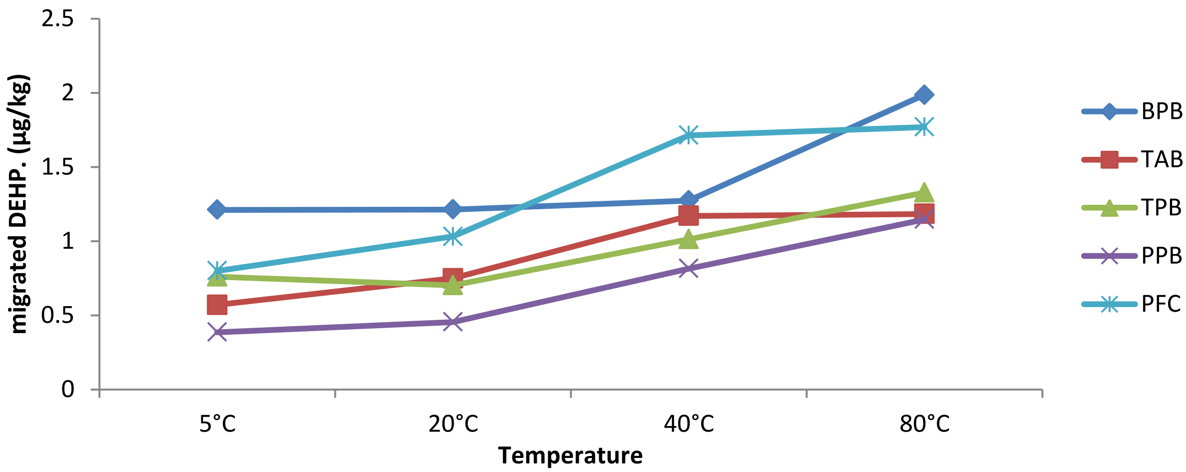


Fig.4.14b. Concentrations of migrated DEHP from the plastic materials for 1hr

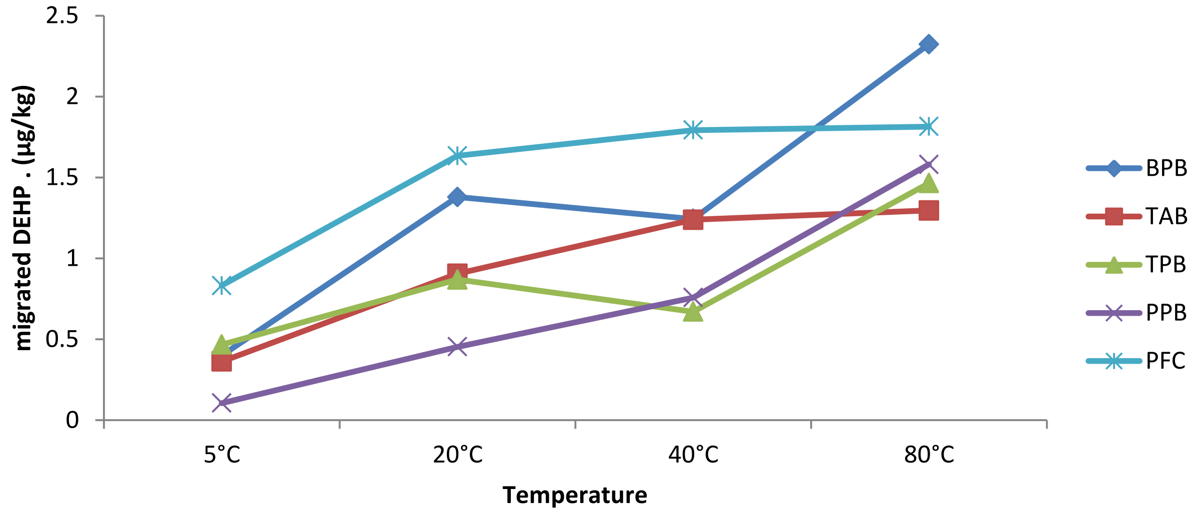


Fig.4.14c. Concentrations of migrated DEHP from the plastic materials for 2hrs

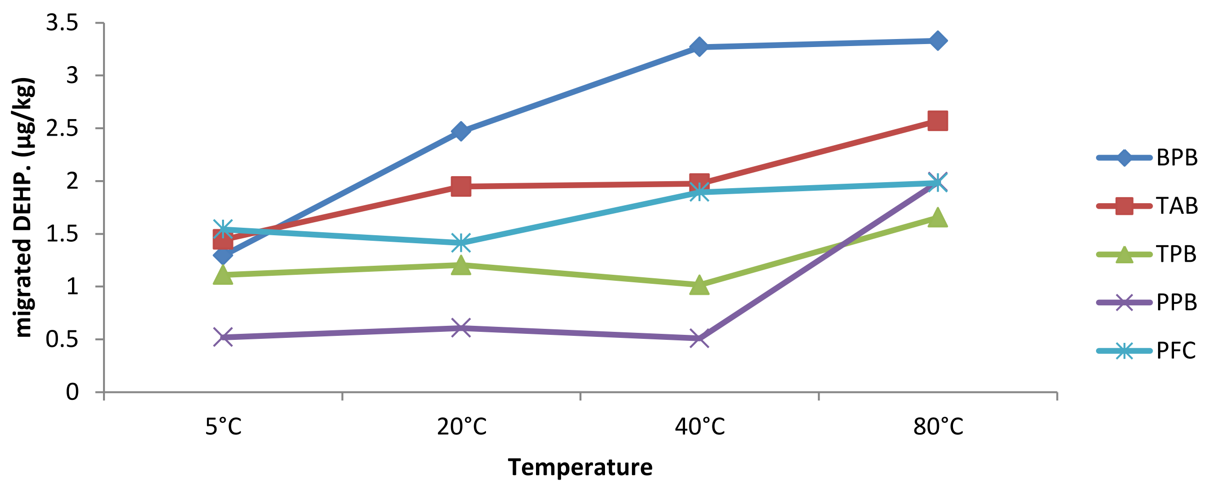


Fig.4.14d. Concentrations of migrated DEHP from the plastic materials for 4hrs

4.3.2.2 Migration of DBP from the plastics at constant exposure times

DBP migration in polyethylene packaging materials at constant exposure times against varying temperatures was also assessed in all the polyethylene packaging materials. DBP being one of the common phthalates found in food and food packages migrated from the packaging materials to the food simulant at varying temperatures. The average mean concentration of the migrated DBP was however lower in concentration than the migrated concentration of the DEHP. This may be due to the difference in molecular structure. Phthalates with long chains such as DEHP are weakly bonded to the packaging materials hence would easily migrate when heat is applied.

Though the concentrations of DBP that migrated from the packaging materials to the food simulant were detected in lower concentrations, there was a linear correlation between the temperature and the migrated phthalate concentrations. The concentrations of the migrated DBP increased gradually as the temperature of the exposure time increased. The **table 4.13** below shows the migrated concentrations of DBP detected in the polyethylene packaging materials after 30 minutes of exposure of the test materials to the food simulant. **Fig. 4.15a** shows a graphical representation of DBP migration detected after the 30 minutes exposure time.

Table4.13. Mean concentrations of migrated DBP for 30 minutes in distilled water

Temperature(°C)	BPB(μg/kg)	TAB(μg/kg)	TPB(μg/kg)	PPB(μg/kg)	PFC(μg/kg)
5	0.03±0.01	0.02±0.01	0.02±0.01	0.05±0.04	0.03±0.01
20	0.06±0.01	0.04±0.02	0.10±0.01	0.06±0.02	0.05±0.01
40	0.16±0.04	0.05±0.01	0.11±0.02	0.24±0.01	0.13±0.04
80	0.23±0.01	0.41±0.13	0.22±0.01	0.29±0.03	0.31±0.05

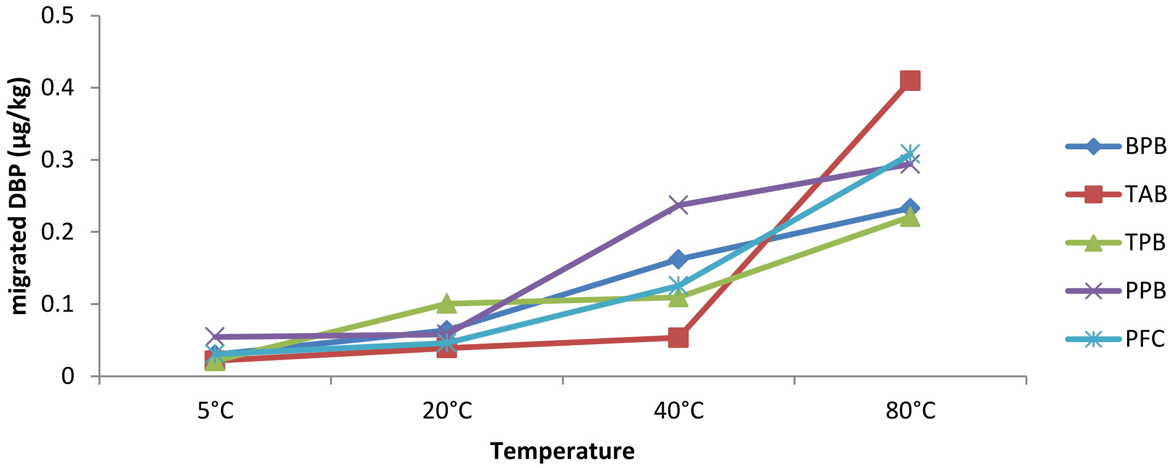


Fig.4.15a. Concentrations of migrated DBP from the plastic materials for 30mins

From **table 4.13** and **fig 4.15a** above, migrated DBP was detected in trace quantities in all the polyethylene packaging materials with a minimum value of 0.02µg/kg detected in the thick polyethylene bags at a temperature of 5°C and a maximum migrated concentration of 0.41µg/kg detected in the take away polyethylene bags at a temperature of 20°C. All the packaging materials had the lowest migrated concentration at the lowest temperature which then increased gradually to the maximum migrated concentrations at the maximum temperature of 80°C. This indicated a linear correlation between the migrated concentrations of the DBP and the exposure temperatures of the packaging materials. This linear correlation between the migrated DBP concentrations and the varied temperatures was observed at the constant exposure times the samples were analysed. This is shown in **tables 4.14-4.16** and **figures 4.15b-d** below;

Table4.14. Mean concentrations of migrated DBP for 1 hour in distilled water

Temperature(°C)	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
5	0.07±0.01	0.05±0.01	0.10±0.03	0.03±0.01	0.04±0.01
20	0.11±0.01	0.04±0.01	0.14±0.02	0.04±0.07	0.14±0.07
40	0.14±0.04	0.06±0.02	0.20±0.07	0.03±0.01	0.16±0.01
80	0.18±0.07	0.17±0.03	0.23±0.04	0.14±0.02	0.18±0.01

Table4.15. Mean concentrations of migrated DBP for 2 hours in distilled water

Temperature(°C)	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
5	0.10±0.01	0.03±0.01	0.10±0.06	0.10±0.22	0.04±0.01
20	0.53±0.09	0.08±0.01	0.65±0.09	0.10±0.10	0.19±0.06
40	0.72±0.14	0.23±0.40	0.56±0.15	0.23±0.09	0.11±0.01
80	1.08±0.11	0.03±0.01	0.76±0.01	0.29±0.17	0.29±0.03

Table4.16. Mean concentrations of migrated DBP for 4 hours in distilled water

Temperature(°C)	BPB(µg/kg)	TAB(µg/kg)	TPB(µg/kg)	PPB(µg/kg)	PFC(µg/kg)
5	0.26±0.07	0.08±0.02	0.68±0.09	0.76±0.07	0.08±0.01
20	0.56±0.08	0.41±0.04	0.81±0.01	0.73±0.04	0.08±0.01
40	0.77±0.09	0.54±0.04	0.89±0.03	1.02±0.15	0.12±0.02
80	1.16±0.34	0.91±0.08	1.00±0.01	1.45±0.59	0.49±0.11

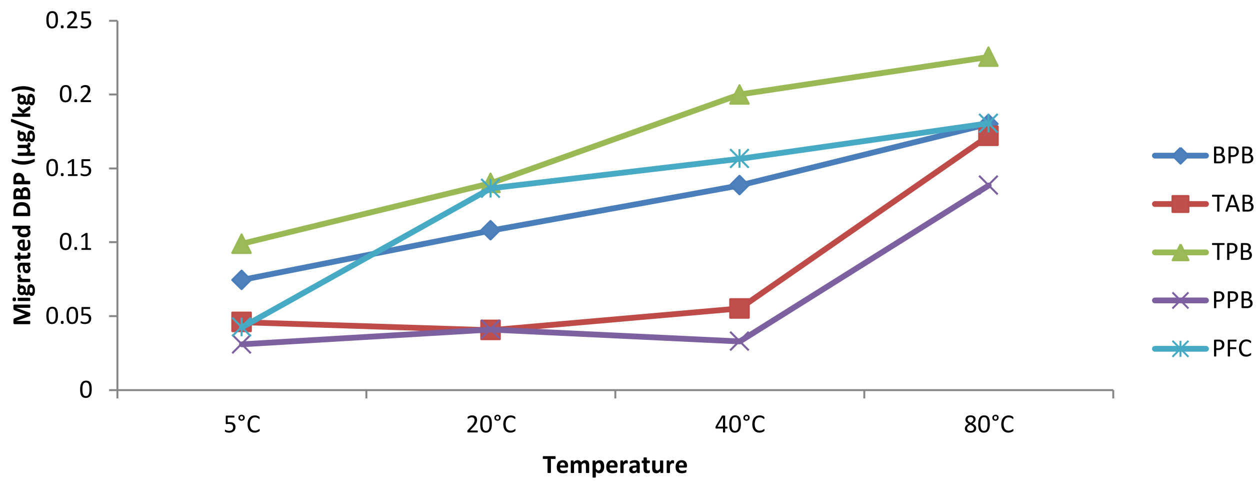


Fig.4.15b. Concentrations of migrated DBP from the plastic materials for 1hr

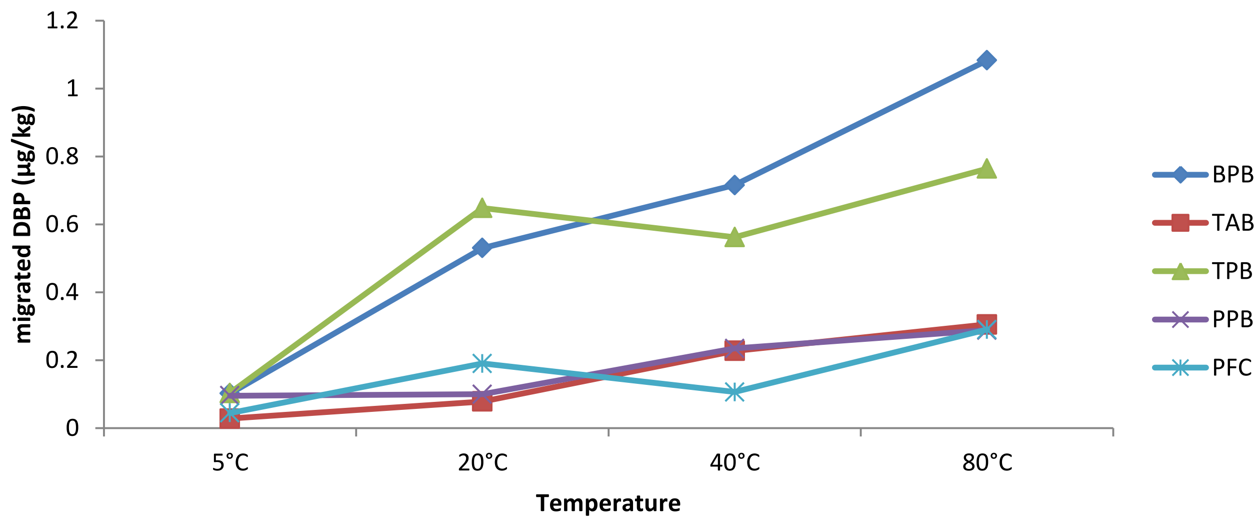


Fig.4.15c. Concentrations of migrated DBP from the plastic materials for 2hrs

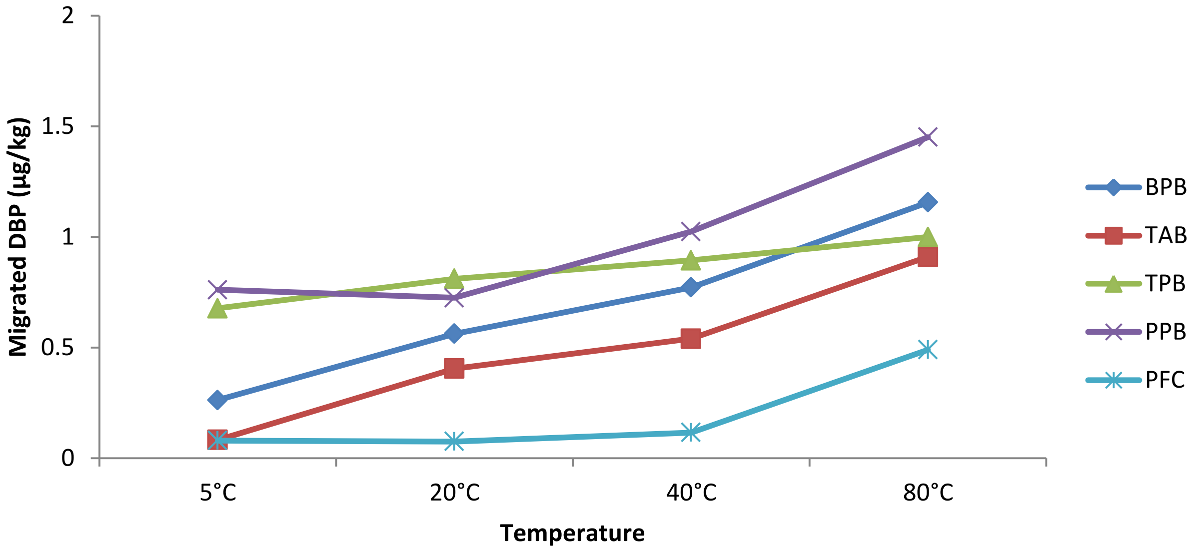


Fig.4.15d. Concentrations of migrated DBP from the plastic materials for 4hrs

4.4 TOTAL PHTHALATES MIGRATED FROM POLYETHYLENE PACKAGING MATERIALS INTO DISTILLED WATER

4.4.1 TOTAL PHTHALATE MIGRATION AT CONSTANT TEMPERATURE

Phthalates in polyethylene packaging materials when exposed to food simulant such as distilled water as observed in this study migrated from the packaging material to the food simulant irrespective of the contact temperature or the contact time. Though there was no any linear correlation observed between the migrated phthalates and the varied exposure times at constant lower temperatures, phthalates in the polyethylene packaging materials however migrated into the food simulant after the required exposure times. The migrated phthalates are shown in **fig 4.16** below. Both DBP and DEHP showed some levels of migration at the various contact times with the 2 hour contact time showing the highest concentration values of DEHP whilst the highest values of migrated concentration of DBP was detected at the 4 hour contact time. Both DBP and DEHP lowest migrated concentrations were however detected at the 30 minutes exposure time. DEP was detected in minute concentration of 0.01µg/kg at the 4hour exposure time.

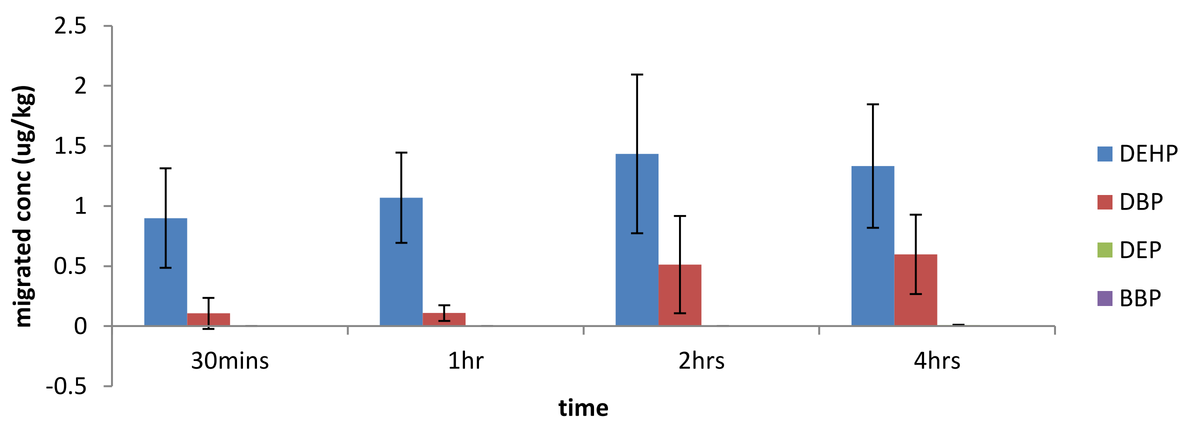


Fig.4.16. Comparison of Phthalates migration from polyethylene packages with time

4.4.2 TOTAL PHTHALATE MIGRATION AT CONSTANT TIME

The migration of phthalates from polyethylene packaging materials into food or food simulant as discussed in the migration trend above has a linear correlation with temperature. The migration of phthalate is dependent on temperature hence the concentrations of migrated phthalates increases as the temperature of exposure between the packaging material and the food or food simulant increases. This is graphically shown in **fig 4.17** below. As shown in the graph, the concentration of the migrated phthalates, i.e. DEHP and DBP increases gradually from the lowest temperature of 5°C to the maximum temperature of 80°C. A study by Batlle and Nerin indicated that temperature increases leads to an increase in the decomposition of additives and breakdown of polymer chains which causes the release of chemicals from the hot plastic surface and hence might have therefore accounted for the increase in migrated phthalate concentration as the temperature increases [6].

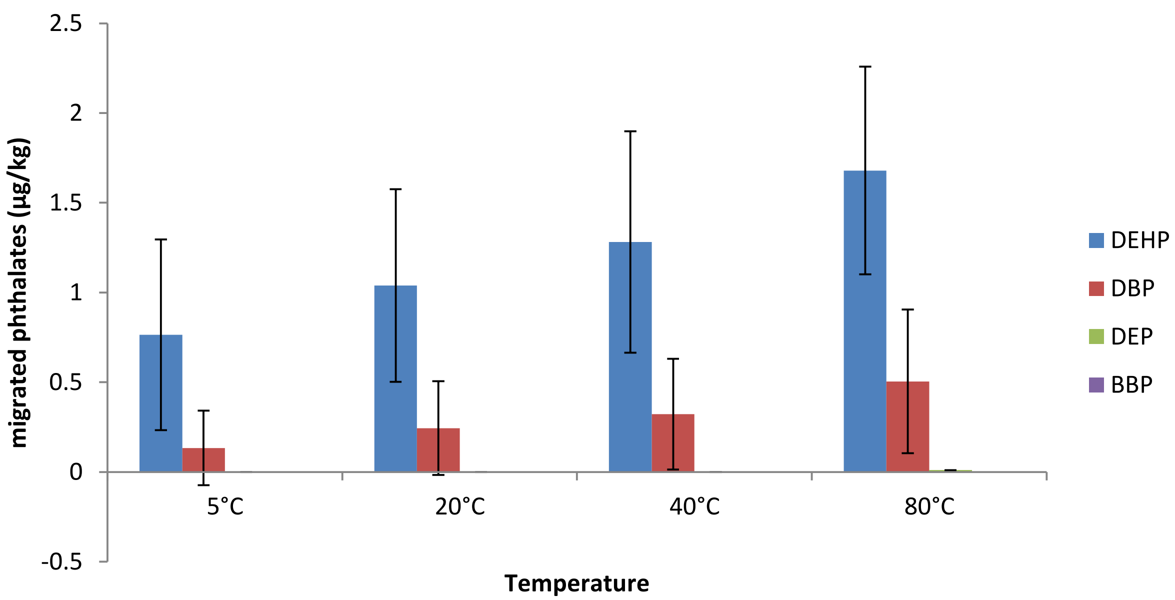


Fig.4.17. Comparison of Phthalates migration from polyethylene packages with temperature

Migration of chemical compounds such as phthalates from packaging polymers to food or food simulants should be evaluated to ensure that the amount of migrating components meet compliance standards set by regulatory agencies.

As observed in this work, temperature increased the diffusion rate/ migration rate of chemical components from the polyethylene packaging materials into food/food simulant. The concentrations of all the migrated phthalates detected in this work are however below the specific migration limits of the various phthalates as stated by European Commission (EU 10/2011). According to the regulation, the specific Migration Limits (SML) of the common phthalates in products intended for food contact is 0.3mg/kg of food simulant for DBP, 30.0mg/kg for BBP and 1.5mg/kg for DEHP. Though the soxhlet extract of the various polyethylene packaging materials showed significant concentrations of the phthalates detected with the exception of BBP which was detected in only one sample, the migrated concentrations of the phthalates from the packaging materials to the aqueous food simulant however showed trace concentrations. For example, the average of the maximum migrated concentrations of the various phthalates was 1.68μg/kg, 0.60μg/kg, and 0.01μg/kg for DEHP, DBP and DEP respectively whilst the average maximum concentrations of the extracts of the various phthalates in the various packaging materials detected were 6.65mg/kg, 8.94mg/kg and 0.06mg/kg for DEHP, DBP and DEP respectively. The predominant phthalate detected in the polyethylene packaging material soxhlet extracts was the DBP whilst DEHP was predominant in the migrated phthalates in the same packaging materials. BBP was detected in only one sample of the packaging materials of the soxhlet extracts but was not detected in any packaging material sample during the migration analysis. Though DEP was detected in trace concentrations in the packaging materials extracts, it was however not detected in the packaging materials during the migration analysis in the aqueous food simulant but at minute concentrations during the longest exposure time and the highest temperature. The low concentrations of the phthalates detected during the migration analysis may be due to the less solubility of these phthalates in water.

4.5 MIGRATION OF PHTHALATES FROM POLYETHYLENE PACKAGES INTO FATTY FOOD SIMULANT (OLIVE OIL)

Most foodstuffs are complex matrixes consisting of a mixture of water, fat, proteins, carbohydrates, vitamins and minerals. Food may also differ in terms of the variety of species, soil conditions and climate. In addition to the chemicals that naturally occur in foodstuff, preservatives, antioxidants, stabilizers, flavors, sweeteners or natural colors may be added when food is processed hence making the analysis of food especially fatty foods in terms of chemical migration difficult.

Food simulating solvents often referred as food simulants have therefore been developed due to the inherent difficulties associated with the direct analysis of migrating species in food. The migrations of plastic chemical species into actual foods other than vegetables have been measured infrequently. The use of a food simulant is the only practical approach that is used in assessing the migration of additives from packaging materials into food. These food simulating materials should therefore adequately reflect the food's chemical and physical properties, i.e. the level of migration from the plastic into the simulant should be similar to that into the food it is simulating and hence the food simulant should permit a relatively simple method of analysis.

Two categories of food simulants have been proposed for fatty foods namely organic solvents such as ethanol, liquid-paraffin, n-heptane, diethyl ether, isooctane and edible fats such as coconut oil, olive oil, sunflower oil and a mixture of synthetic triglycerides. The use of edible fats as fatty foods simulant has been more successful than organic solvents, however not entirely satisfactory [44]. The analysis of phthalates migration from polyethylene packaging materials into food was therefore carried out using olive oil as described above in the test method.

The percentage recoveries of the spiked samples are shown in **table 4.1**. The percentage recoveries of the spiked samples of the olive oil were however affected by the high concentration values detected in the sample blanks. This therefore confirmed the several research works that have reported significant concentrations of phthalates especially DEHP in olive oil. For example, F. Lacoste in a study on the undesirable substances in vegetable oils reported concentrations of DEHP greater than 1mg/kg in virgin olive oil and other vegetable oils [45].

4.5.1 MIGRATION OF PHTHALATES FROM POLYETHYLENE PACKAGING MATERIALS INTO OLIVE OIL AT CONSTANT TEMPERATURE

Table 4.17 shows the various concentrations of the various phthalates migrated from the polyethylene food packages (PFC). DEHP which was detected in all the blank samples was the predominant phthalate detected followed by DEP and then DBP. Whereas DEP was detected in minute concentrations at the highest temperature (80°C) and the longest exposure time (4hrs) in the aqueous food simulant, it however was detected in almost all the samples, some with significant concentrations in the olive oil. The migrated concentrations of DEHP ranged from 0.69mg/kg to 1.95mg/kg at the highest temperature and longest exposure time. DEP migrated concentrations however ranged from not detected to a concentration of 1.77mg/kg. DBP which was one of the predominant migrated phthalates detected in the aqueous food simulant was also detected in significant concentrations in the fatty food simulant (olive oil) ranging from not detected to 1.43mg/kg. BBP was not detected in any of the polyethylene packaging materials. The same was observed in the phthalate migration into the aqueous food simulant. The migrated phthalate concentrations detected in the olive oil however showed very high concentrations compared to the migrated phthalate concentrations detected in the aqueous food simulant. This may be due to the high affinity for fats by phthalates. The graphical representation of the migrated phthalates verses the exposure times at constant temperature are shown in **fig4.18-4.20**.

Table.4.17 Average concentrations of migrated phthalates in polyethylene food container (PFC)

DEHP	5°C (mg/kg)	20°C (mg/kg)	40°C (mg/kg)	80°C (mg/kg)
30 mins	0.69±0.03	0.89±0.04	1.19±0.02	1.29±0.02
1hr	0.76±0.09	1.00±0.02	1.71±0.55	2.02±0.74
2hrs	1.07±0.06	1.19±0.06	1.61±0.16	1.95±0.14
4hrs	1.22±0.08	1.54±0.06	1.60±0.05	1.68±0.05
DEP				
30 mins	ND	0.05±0.02	0.59±0.06	0.89±0.03
1hr	0.04±0.01	0.28±0.04	0.43±0.06	0.71±0.04
2hrs	0.32±0.03	0.49±0.08	0.79±0.04	1.04±0.02
4hrs	0.61±0.16	0.78±0.19	1.55±0.17	1.78±0.06
DBP				
30 mins	ND	ND	0.03±0.01	0.04±0.01
1hr	0.04±0.01	0.09±0.01	0.16±0.01	0.18±0.05
2hrs	0.08±0.01	0.16±0.04	0.27±0.02	0.50±0.02
4hrs	0.14±0.02	0.43±0.10	1.14±0.01	1.43±0.28

ND=Not detected

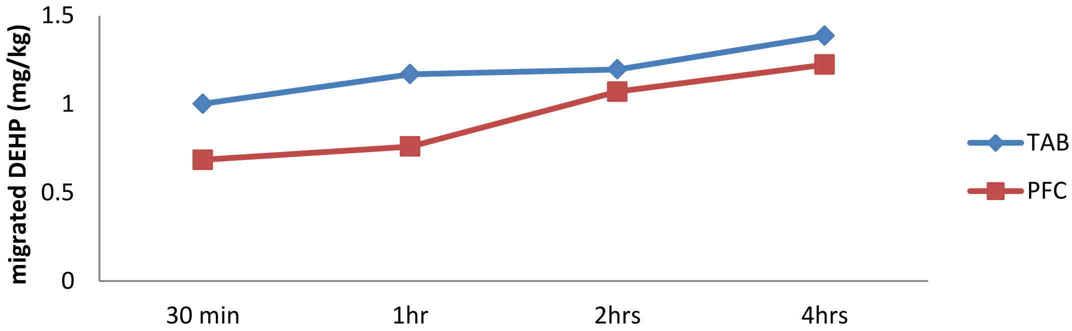


Fig4.18. Concentrations of migrated DEHP at 5°C in olive oil

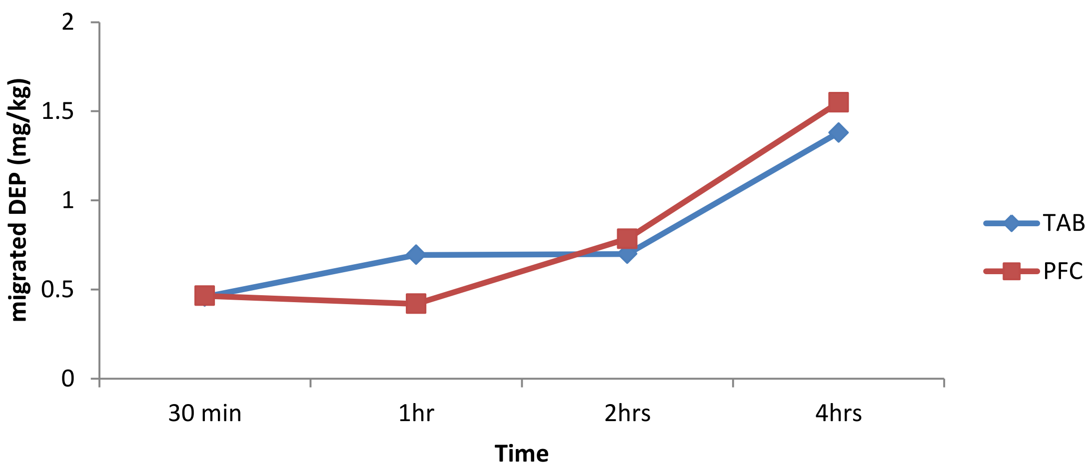


Fig.4.19. Concentrations of migrated DEP at 40°C in olive oil

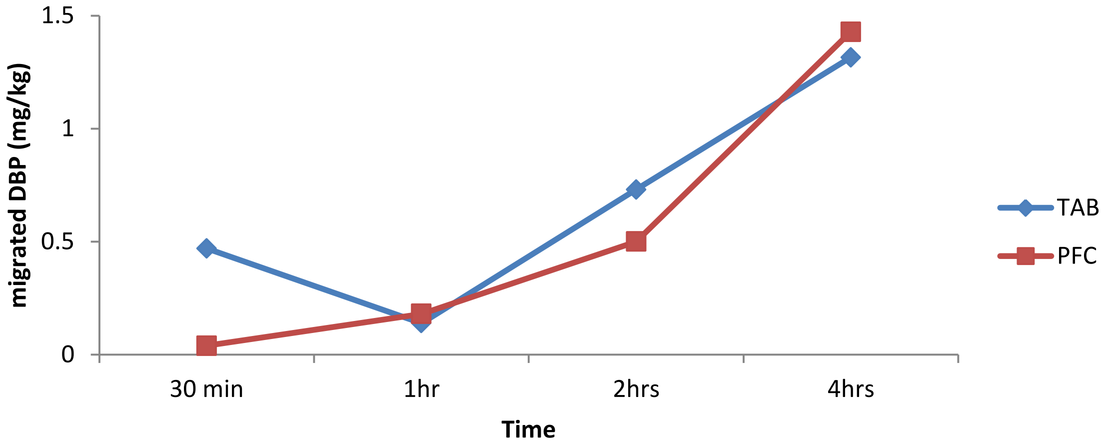


Fig.4.20. Concentrations of migrated DBP at 80°C in olive oil

From **fig 4.18-4.20**, it is observed that the migrated phthalates concentration increases as the exposure time increases from 30 minutes to 4 hours. For instance, the concentration of migrated DEP increased from not detected at the 30minutes exposure time to 0.61mg/kg of the 4hr exposure time at the 5°C constant temperature and also from 0.89mg/kg at the 30minutes exposure time to 1.78mg/kg of the 4hr exposure time at the 80°C. Thus the rate of migration increased with increasing exposure time hence indicating a correlation between the migrated phthalates and the exposure times. This trend of phthalate migration with contact time was observed in all the migrated phthalates in all the packaging materials. A study on the migration of plasticizers from PVC films into food by Mercer A. reported that the levels of phthalates found in retail foods wrapped in phthalate plasticized films contained 0.1ppm -14.1ppm DBP and 0.06ppm – 2.4ppm DEHP [44]. The migrated phthalate concentrations reported in this work therefore are similar to what was reported by Mercer.

4.5.2 MIGRATION OF PHTHALATES FROM POLYETHYLENE PACKAGES INTO OLIVE OIL AT CONSTANT CONTACT TIME

Average concentrations of the various phthalates migrated from the take away polyethylene bags are shown in **table 4.18** below. Take away polyethylene bags are the common plastics use to wrap most raw and ready to eat foods on the Ghanaian market. Similar to what was observed in the migration of phthalates in the polyethylene food containers, the predominant phthalate was DEHP followed by DEP and then DBP. BBP was not detected in any of the samples.

From table 4.18, it is observed that the migrated concentrations of the phthalates increased as the temperature increased from 5°C to 80°C. For example, the migrated concentration of DBP at 5°C increased from0.02mg/kg to 0.47mg/kg at 80°C for the 30 minutes contact time and also from 0.08mg/kg at 5°C to 1.32mg/kg at 80°C for the 4hr contact time. This trend of increase in phthalate migration with increasing temperature was observed in all the polyethylene packaging materials analysed as shown in **tables 4.17** and **4.18.**

Fig 4.21 -4.23 Shows the observed concentrations of the various migrated phthalates with varying temperature

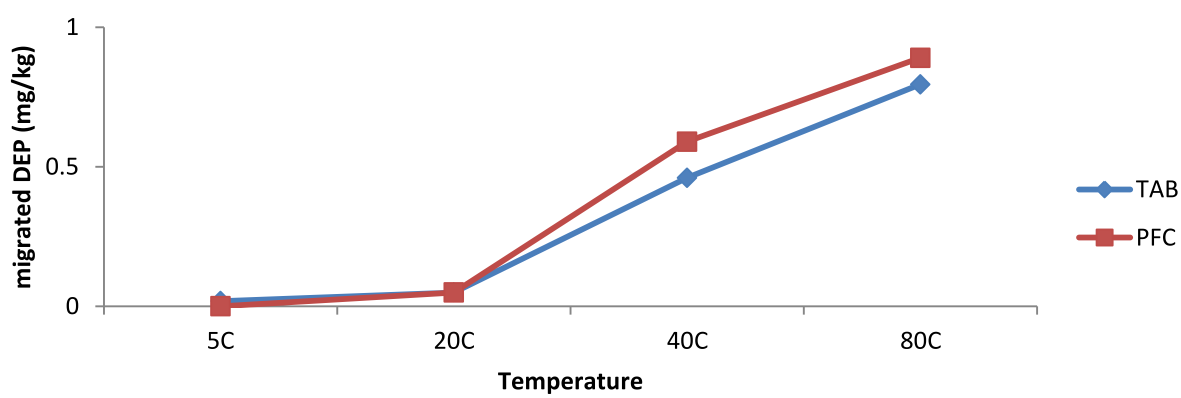


Fig. 4.21 Concentrations of migrated DEP into olive oil for 30 mins

Table.4.18 Average concentrations of migrated phthalates in take away bags (TAB)

DEHP	5°C (mg/kg)	20°C (mg/kg)	40°C (mg/kg)	80°C (mg/kg)
30 mins	1.00±0.02	1.37±0.23	0.54±0.20	1.01±0.01
1hr	1.17±0.02	1.38±0.25	1.74±0.20	1.15±0.03
2hrs	1.20±0.01	1.30±0.07	1.35±0.10	1.57±0.06
4hrs	1.39±0.10	1.39±0.04	1.48±0.20	1.48±0.07
DEP				
30 mins	0.02±0.04	0.05±0.02	0.46±0.06	0.80±0.06
1hr	0.39±0.04	0.54±0.08	0.70±0.06	0.77±0.06
2hrs	0.49±0.02	0.57±0.08	0.70±0.06	0.88±0.05
4hrs	0.52±0.02	0.67±0.06	1.38±0.10	1.58±0.06
DBP				
30 mins	0.02±0.01	0.04±0.01	0.36±0.02	0.47±0.06
1hr	0.03±0.01	0.13±0.01	0.03±0.01	0.14±0.01
2hrs	0.05±0.03	0.31±0.05	0.50±0.10	0.73±0.12
4hrs	0.08±0.02	0.54±0.18	1.06±0.03	1.32±0.03

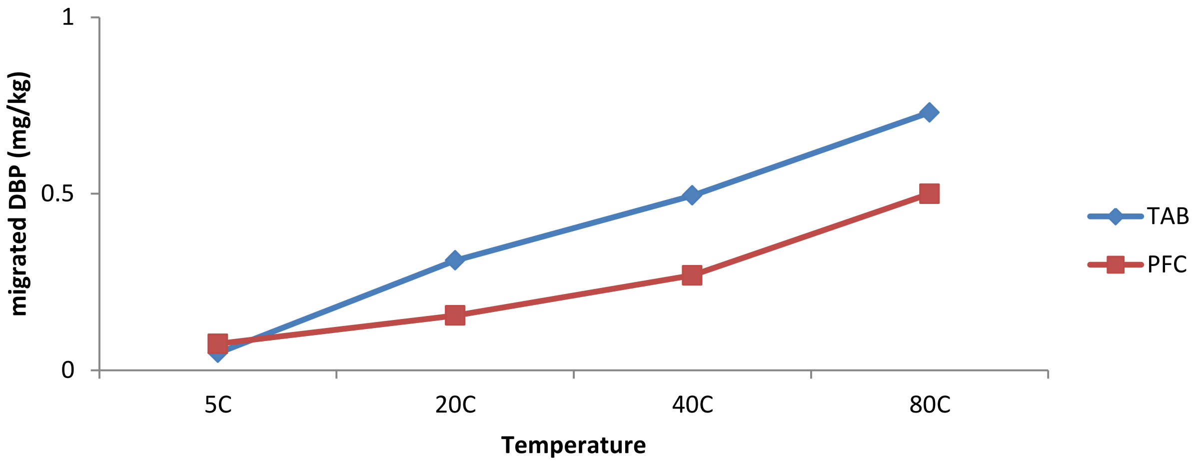


Fig.4.22. Concentrations of migrated DBP into olive oil for 2hrs

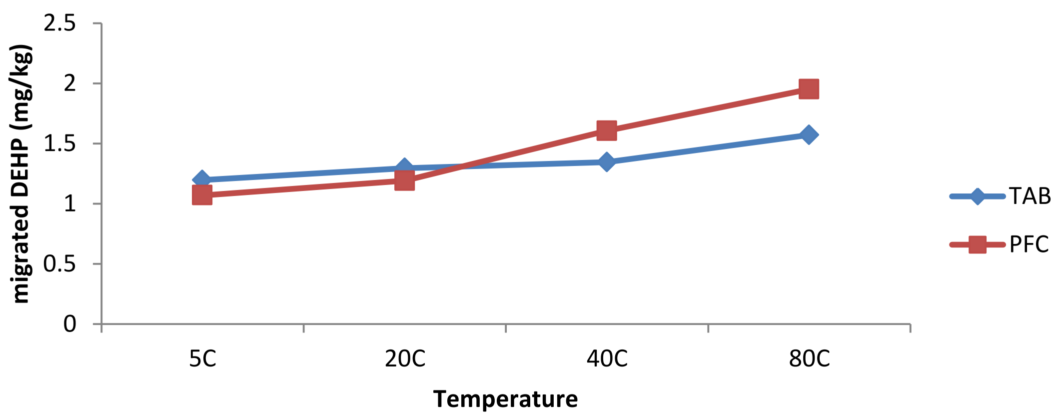


Fig.4.23. Concentrations of migrated DEHP into olive oil for 2hrs

The migration of phthalates to food/food simulants from packaging materials under different storage conditions for the past decade have been reported by several researchers such as castle et al., Lau and wong , Goulas et al., etc. For example, a study by Xu et al. evaluated the migration of 8 phthalates from plastics in cooking oil and mineral water under different storage conditions and reported that the content of the 8 phthalates were always higher in the cooking oil than the mineral water [28]. DEHP and DBP according to Xu et al. were the dominant phthalates in the cooking oil migration whilst DBP and DINP were dominant in the mineral water migration hence similar to what is reported in this work. In addition, Badeka and Kontominas in a study on the effects of heating on the migration of dioctyladipate (DOA) from food grade PVC films into olive oil and distilled water reported that the migration was always higher in the olive oil compared to the distilled water under similar conditions [28].

As observed in **fig 4.18-4.23** above, the concentrations of migrated phthalates to olive oil are not only higher in the fatty food simulant but also increases with increased in both temperature and exposure time.

CHAPTER FIVE

5 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Packaging materials and systems for foods contain many substances which can migrate into food during processing or storage. Some of these chemicals can be present in foodstuff even though their introduction may not be expected from normal practices and procedures. The major concern regarding the safety of these food contact materials is that, the effects of prolonged exposure to humans are still poorly known. This thesis therefore reviewed the migration of chemical compounds (phthalates) as influenced by temperature and exposure times using distilled water as aqueous food simulant and olive oil as fatty food simulant. This research was to identify and quantify phthalates in polyethylene packaging materials and also quantify by specific migration (SM), phthalates in polyethylene food packaging materials.

The four phthalates under investigation were all detected in significant quantities in the extracts of all the polyethylene packaging materials with the exception of BBP which was detected in only one out of the 25 samples analysed. The mean concentration of the phthalates in the packaging materials detected were 6.65mg/kg for DEHP, 8.94mg/kg for DBP, 1.69mg/kg for DEP and 0.06mg/kg for BBP.

The concentration values of all the phthalates detected in the aqueous food simulant in this study were in accordance with the current EC regulation for the migration of phthalates in food contact materials (FCM). The regulation (EU) No. 10/2011 states 1.5mg/kg as the specific migration limit (SML) of food simulant for DEHP, 0.3mg/kg for DBP and 30.0mg/kg for BBP of products intended for food contact. The concentration of all the migrated phthalates obtained from the polyethylene packaging materials in the distilled water were below the SML stated above. DEHP which was the dominant phthalates migrated in the distilled water had its highest migrated concentrations of 1.68µg/kg at the highest temperature and 1.43µg/kg at the 2 hours exposure time. DBP on the other hand had its highest migrated concentration of 0.60µg/kg at the four hour exposure time and 0.50µg/kg at the highest temperature of 80°C. However the migrated concentrations of the phthalates in the olive oil had some of its concentration levels exceeding the SML stated in the regulation (EU) 10/2011. DEHP, which was the predominant phthalate in the olive oil migration, had 28.13% of the migrated concentrations exceeding the SML stated above. DBP also had 34.38% of the detected migrated concentrations exceeding the stipulated SML for DBP for food contact materials.

The concentrations of the migrated phthalates detected increased with temperature in all the packaging materials both in the aqueous food simulant and the fatty food simulant. There was a correlation between the increase in phthalate migration and increasing temperature in both food simulants. The migration of phthalates during the various exposure times in the aqueous food simulant though was observed, there was no linear correlation between the migrated phthalate concentration and the exposure time observed where as a linear correlation was observed between the various migrated phthalates and the exposure times in the olive oil migration.

Though the concentrations of migrated phthalates detected in the aqueous food simulant were all below the specific migration limit of the various phthalates as stated by the European regulation (EU) No. 10/2011, the migrated concentrations of DEHP and DBP had 28.13 and 34.38% respectively exceeding the SML for polyethylene food contact materials. The migrated phthalate concentrations found in the fatty food simulant coupled with the ubiquitous nature of phthalates in the environment and several exposure routes, there is the need for more research work to be carried out on phthalates to ascertain the adverse health effects and the possible accumulation of these phthalates at different exposure routes so that regulatory bodies can enact legislation to help curb it use in the manufacturing process especially in the production of food contact materials.

5.2 RECOMMENDATIONS

1. Further studies on phthalate migration from polyethylene packaging materials should be carried out at temperatures beyond 100°C.
2. Phthalates migration on other packaging materials such as polystyrene sold on the Ghanaian market and used in the food industry should be carried out.
3. Further studies should be carried out to identify and quantify other pollutants such as Bisphenol A and Perfluorinated compounds (PFC'S) common in plastic food packages.
4. Analysis of phthalates in olive oil and other common vegetable oils sold on the Ghanaian market and the effect of packaging on the oil should be carried out.
5. The primary purpose of food packaging is to maintain safety, wholesomeness and quality of food, hence regulatory institutions should carry out more research on these pollutants to help enact legislations in order to regulate the type and amount of plasticizers used during the manufacturing of these food packaging materials

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APPENDICES

Appendix 1A, Categorization of polyethylene packaging materials

PRODUCT	MELTING POINT RANGE	CATEGORY
Thick Plain polythene bags (TPB)	106°C -108°C	LDPE
Take Away polythene bags (TAB)	108°C -113°C /128°C -130°C	HDPE/LDPE
Polythene plastic Bottles (PPB)	129°C-130°C	HDPE
Black polythene Bags (BPB)	106°C -113°C / 128°C -130°C	LDPE/HDPE
Polythene Food Containers (PFC)	128°C -130°C	HDPE

Appendix 1B, Melting points of polyethylene packaging materials

Category\Batch	1	2	3	4	5
BPB	106°C-108°C	106°C-107^{0}°C	129°C-130°C	128°C-130°C	109°C-113°C
TPB	107°C -110°C	105°C -107°C	106°C -108°C	110°C -112°C	105°C -107°C
TAB	108°C -112°C	128°C -129°C	109°C -113°C	129°C -130°C	129°C -130°C
PFC	129°C -130°C	128°C -129°C	129°C -130°C	128°C -130°C	127°C -129°C
PPB	128°C -129°C	129°C -130°C	129°C -130°C	129°C -131°C	128°C -130°C

Appendix2A. Mean concentration of migrated DEHP at 20°C in distilled water

Samples	30 min	1hr	2hrs	4hrs
BPB (µg/kg)	0.83±0.02	0.99±0.12	0.76±0.01	1.47±0.01
TAB (µg/kg)	0.90±0.12	1.18±0.02	1.09±0.13	0.95±0.01
TPB (µg/kg)	0.89±0.21	0.70±0.22	0.87±0.21	1.20±0.03
PPB (µg/kg)	0.05±0.22	0.45±0.20	0.45±0.03	0.61±0.13
PFC (µg/kg)	1.31±0.21	1.03±0.41	1.63±0.20	1.41±0.23

Appendix2B. Mean concentration of migrated DEHP at 40°C in distilled water

Samples	30mins	1hr	2hrs	4hrs
BPB (µg/kg)	0.96±0.34	1.27±0.03	2.24±0.10	2.27±0.10
TAB (µg/kg)	1.16±0.20	1.17±0.20	1.44±0.02	1.06±0.40
TPB (µg/kg)	1.06±0.31	1.01±0.11	0.67±0.02	1.01±0.02
PPB (µg/kg)	0.26±0.13	0.59±0.31	0.76±0.33	1.89±0.03
PFC (µg/kg)	0.56±0.23	1.71±0.20	1.79±0.24	0.51±0.15

Appendix2C. Mean concentration of migrated DBP at 5°C in distilled water

Samples	30mins	1hr	2hrs	4hrs
BPB (µg/kg)	0.03±0.01	0.07±0.01	0.10±0.01	0.26±0.01
TAB (µg/kg)	0.41±0.11	0.05±0.01	0.03±0.01	0.41±0.22
TPB (µg/kg)	0.10±0.11	0.23±0.21	0.76±0.02	0.08±0.02
PPB (µg/kg)	0.49±0.23	0.03±0.01	1.16±0.05	0.76±0.12
PFC (µg/kg)	0.03±0.01	0.04±0.03	0.04±0.01	0.08±0.01

Appendix2D. Mean concentration of migrated DBP at 20°C in distilled water

Samples	30mins	1hr	2hrs	4hrs
BPB (µg/kg)	0.06±0.04	0.18±0.12	0.53±0.21	0.56±0.21
TAB (µg/kg)	0.04±0.01	0.04±0.01	0.08±0.01	0.05±0.02
TPB (µg/kg)	0.11±0.23	0.14±0.22	0.09±0.02	0.65±0.32
PPB (µg/kg)	0.06±0.03	0.06±0.01	0.85±0.02	0.73±0.30
PFC (µg/kg)	0.07±0.02	0.10±0.20	0.12±0.01	0.18±0.31

Appendix2E. Mean concentration of migrated DBP at 40°C in distilled water

Samples	30mins	1hr	2hrs	4hrs
BPB (µg/kg)	0.12±0.20	0.18±0.20	0.83±0.28	0.66±0.24
TAB (µg/kg)	0.02±0.32	0.05±0.23	0.83±0.02	0.33±0.13
TPB (µg/kg)	0.22±0.23	0.20±0.01	0.56±0.20	0.89±0.30
PPB (µg/kg)	0.03±0.01	0.03±0.01	0.70±0.04	0.17±0.15
PFC (µg/kg)	0.13±0.11	0.16±0.03	0.19±0.13	0.12±0.02

Appendix3A. Mean concentrations of migrated DEHP for 2hr in distilled water

Samples	5°C	20°C	40°C	80°C
BPB (µg/kg)	0.40±0.29	1.38±0.34	1.24±0.32	2.32±0.03
TAB (µg/kg)	0.36±0.01	0.91±0.08	1.24±0.33	1.30±0.23
TPB (µg/kg)	0.47±0.01	0.87±0.01	0.68±0.10	1.47±0.30
PPB (µg/kg)	0.11±0.05	0.45±0.12	0.76±.20	1.58±0.05
PFC (µg/kg)	0.83±0.02	1.63±0.06	1.79±0.20	1.81±0.24

Appendix3B. Mean concentrations of migrated DEHP for 4hr in distilled water

Samples	5°C	20°C	40°C	80°C
BPB (μg/kg)	1.29±0.71	2.47±0.51	3.27±0.49	3.33±0.38
TAB (μg/kg)	1.45±0.21	1.95±0.55	1.98±0.30	2.57±0.33
TPB (μg/kg)	1.11±0.19	1.20±0.53	1.01±0.55	1.65±0.23
PPB (μg/kg)	0.52±0.22	0.61±0.22	0.51±0.22	1.99±0.21
PFC (μg/kg)	1.54±0.35	1.41±0.55	1.89±0.54	1.98±0.27