PREVALENCE OF ORGANO-MICROBIAL ENTITIES IN SELECTED COMMERCIAL FOODS AND FOOD WRAPPERS

by

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DECLARATION

I, Ndingoho Masakona, hereby declare that the research project entitled "Prevalence of organo-microbial entities in selected commercial foods and food wrappers" was carried out by me as part of the funded research project by the South African National Research Foundation (NRF). I also declare that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. The dissertation has not been submitted or will not be submitted to a university or any institution for the award of a degree.

Signed (Author)_____ Date_____

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ABSTRACT

Phthalate esters (PEs) belong to a class of organic compounds used as plasticisers in plastic materials such as polyvinyl chloride (PVC), polypropylene (PP), polyethylene terephthalate (PET) and so on, including those used in the food packaging industry. Phthalate plasticisers are not chemically bound to plastic materials and hence, migrate into items such as foodstuffs they house. The study aimed at investigating the prevalence of selected phthalate esters from plastic wrappers into food as well as the presence of food and/or pathogenic micro-organisms.

Plastic-wrapped cheese, vienna sausages and polony samples purchased from commercial stores in the four regions of Pretoria (Tswane), South Africa, were analysed for the presence of plasticisers; di-2-ethylhexyl adipate (DEHA), di-n-butyl phthalate (DnBP), benzyl-butyl phthalate (BBP), di-butyl phthalate (DBP) and di-methyl phthalate (DMP). Soxhlet extraction using hexane with florisil column cleanup was carried out. Analysis of PEs was by Gas Chromatography-Flame Ionization Detection (GC-FID). Microbiological investigations were performed using standard methods.

The concentrations of PEs detected in food samples ranged from below detection limit (bdl) to 4.7003 µg/kg. However, DBP, DMP and BBP were predominantly present with more PEs detected in cheese compared to polony and vienna. In polony samples, DBP levels ranged from 0.0412 to 0.611µg/kg, in cheese, ranged from 0.049 to 0.256 µg/kg and in vienna DBP ranged from 0.074 to 0.209 µg/kg. The phthalate DMP ranged from 0.072 to 4.700 µg/kg in cheese, 0.056 to 0.241 µg/kg in polony and 0.092 to 0.816 µg/kg in vienna. The DEHA detected in cheese and polony was 0.120 µg/kg and 0.075 µg/kg respectively and no DEHA was detected in vienna sausages.

For microbiological analysis, the total microbial activity (TMA) ranged from 6.8×10^4 to 1.03×10^8 cfu/g; coliforms ranged from no growth to 2.62×10^6 cfu/g; yeast ranged from no growth to 1.49×10^7 cfu/g; and mould ranged from no growth to 9.2×10^4 cfu/g. The results revealed that microbial activity was high in each sample type but revealed the absence of pathogens. Results revealed incidences of PEs in foods

wrapped or packaged in plastics, which gave cause for concern and showed the need for proper monitoring and inspection of the levels of organo-microbial entities in the South African food wrapped in plastic wrappers.

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ABBREVIATIONS AND ACRONYMS

ABS	Acrylonitrile butadiene styrene
AGD	Anogenital distance
ASTM	American Society for Testing Materials
ATBC	Acetyltributyl citrate
BBP	Benzyl-butyl phthalate
BCP	Butyl cyclohexyl phthalate
BDP	Butyl decyl phthalate
BGLB	Brilliant green lactose bile
BP	Baird-Parker
BS	Bismuth sulfite
CE	Cheese East
CEC	Commission of the European Communities
CERHR	Center for the Evaluation of Risks to Human Reproduction
CN	Cheese North
CS	Cheese South
CW	Cheese West
DAP	Diallyl phthalate
DBP	Dibutyl phthalate
DCP	Dicyclohexyl phthalate
DEHA	Di-2-ethyl hexyl adipate
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DIHpP	Diisoheptyl phthalate
DIHxP	Diisohexyl phthalate
DINP	Diisononyl phthalate
DIOP	Diisooctyl phthalate
DIUP	Diisoundecyl phthalate
DMP	Di-methyl phthalate
DnBP	Di-n-butyl phthalate

DNHP	Di-n-hexyl phthalate
DNOP	Di(n-octyl) phthalate
DNP	Di-n-pentyl phthalate
DOA	Dioctyladipate
DOP	Di-octyl phthalate
DPP	Di-n-propyl phthalate
DTUP	Ditridecyl phthalate
DUP	Diundecyl phthalate
EACs	Endocrine-active chemicals
ECD	Electron capture detector
ECMO	Extracorporeal membrane oxygenation
EDC's	Endocrine Disrupting Compounds
EPA	Environmental Protection Agency
EU	European Union
EU TDI	European Union tolerable daily intake
EVA	Ethylene vinyl acetate
EVOC	Ethylene vinyl alcohol
FCD	Foodstuffs, Cosmetics and Disinfectants
FDA	Food and Drug Administration
GC	Gas Chromatography
GC/MS	Gas chromatography–mass spectrometry
GC-FID	Gas chromatography-flame ionisation detection
HE	Hektoen enteric
HNP	High nitrile polymers
HPLC	High performance liquid chromatography
IFST	Institute of Food Scince and Technology
ISTD	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
LLE	Liquid–liquid extraction
MAE	Microwave-assisted extraction
MBP	Mono-n-butyl phthalate
MBzP	Monobenzyl phthalate
MEHP	Mono-2-ethylhexyl phthalate
MEP	Monoethyl phthalate

MiNP	Monoisononyl phthalate
ODP	n-Octyl n-decyl phthalate
OML	Overall migration limit
PA	Polyamides
PAH	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction
PE	Polyethylene
PE	Polony East
PET	Polyethylene terephthalate
PLE	Pressurised liquid extraction
PN	Polony North
PP	Polypropylene
ppm	Parts per million
PS	Polony South
PS	Polystyrene
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
PVdC	Polyvinylidene chloride
PW	Polony West
Py-GC	Pyrolysis-gas chromatography
RfD	Reference dose
RH	Relative humidity
SANAS	South African National Accreditation System
SB	Styrene butadiene
SC	Selenite cystine
SMLs	Specific migration limits
SPE	Supercritical fluid chromatography
SPME	Solid-phase micro-extraction
TMA	Total microbial activity
TSAYE	Trypticase soy agar yeast extract
TT	Tetrathionate
TTDI	Total tolerable daily intake
UK	United Kingdom

US	United States
VE	Vienna East
VN	Vienna North
VRBL	Violet red bile lactose
VS	Vienna South
VW	Vienna West
WHO	World Health Organisation
XLD	Xylose lysine desoxycholate

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AN OVERVIEW OF OUTLINE OF THE DISSERTATION

The outline below provides an indication of the different chapters in this study and assists the reader in understanding the flow of the investigation procedure that was followed and the main sections that are dealt with in each chapter. The dissertation consists of six chapters.

Chapter 1 provides the background together with the motivation of the study. The purpose of this investigation is highlighted and the issues of phthalate esters are briefly explored. The study areas, the hypothesis, aims and objectives of the study are discussed.

The next chapter, Chapter 2, explores the literature relevant to the study. The characteristics of phthalate esters, their sources and uses are described in detail. The chemical and environmental properties of phthalate esters are stipulated as well as the main risks of human exposure to these environmentally dangerous chemicals. It presents international and national standards with regard to phthalate esters. Finally, it discusses trends in phthalate esters research, the shortcomings of the different research and lays the theoretical foundations for the study.

Chapter 3 describes the methodology and experimental procedures used in the study. It also describes the sampling procedure and the data-collection procedure together with the study area.

Chapter 4, presents an analysis of the results obtained, and provides illustrations and discussion of the results. It also compares the results with international and national standards.

Finally, chapter 5 and 6 discusses the results, highlighting the trends observed and seeks to answer the research problem, and to accept or reject the hypothesis. It suggests possible solutions and recommendations and provides conclusions to the study.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Over the past decades, the safety of food to consumers has become an issue. The food we eat is sometimes non-sterile. They contain different microbial associations which are different depending on which microorganisms were able to gain access and how they grow, exist and interact in the food (Adams and Moss, 2000). The microorganisms which are present in food, originate from the normal microflora found in food, and those introduced during processing, harvesting, storage, and distribution.

Food has a relationship with the transmission of disease. Therefore, it is essential to have regulations on food hygiene. Food microbiology can be defined as an applied science in which the food microbiologist's primary purpose is to assist in ensuring the supplying of consumers with safe food (Adams and Moss, 2000). Food Microbiology reinforces the significance of this crucial element of food industry

The use of appropriate packaging technology to reduce food losses and provide safe and healthy food products has always been the focus of food packaging (Lee *et al*., 2008). The need for proper packaging in the food industry has grown over decades. Food packaging is one of the most fundamental aspects in the food industry since health and safety is the primary objective. According to Wikipedia (2011: accessed 23rd March 2011), food packaging can be defined as the enclosing of food which requires protection from tampering, whether by physical, chemicals, or biological needs, also bearing any nutrition information on the food consumed. The role of packaging is to carry, protect or preserve food and thus reduce food waste. This also assists the world's preservation of all its resources through the prevention of product spoilage and wastage (Potter and Hotchkiss, 1995).

There are different uses of food packaging, which includes usage as a form of communication. This helps the customer to be able to identify the product because of the labelled text and graphics. This can also communicate to the consumer on the usage of the product as the packages also feature nutritional information of the packaged food (Hanlon *et al*., 1998 and Wikipedia, 2011: accessed 23 March 2011).

The packaging used depends on the type of food packaged; for example, liquids or solids. Packaging durability is another factor in containment. This involves the packaged food enduring the process of transportation from the processing facility, to the supermarket and finally, to the home for the consumer (Wikipedia, 2011: accessed 23 March 2011). Packaging can be useful in relation to environmental issues. Most of the packaging materials used are reusable. This then minimises contamination of the environment (Steven and Hotchkiss, 2003).

There are different types of food packaging used. They can be divided into primary packaging; which includes, the main package, that holds the processed food; secondary packaging, which combines the primary packages into one box; and finally, the tertiary packaging, which combines all of the secondary packages into one pallet. For primary packaging, potato chips packaged in a bag can be an excellent example. Boxes serve as secondary packaging, and a good example is the packaging of cereal. Tertiary packaging can be illustrated with wrappers commonly used to cover boxes on the pellet for transportation (Bourque, 2003; Chinnan and Cha, 2003).

There are also various materials used in the packaging of food. These include metal cans, glass containers, plastics, paper and paperboard packaging. Metal cans are used for noncarbonated drinks such as liquid coffee, tea, and sports drinks and carbonated beverages such as soft drinks and beer (Page *et al*., 2003). Metal cans perform the following basic function: preserve and protect the product, resist chemical reactions, hold out the handling and processing conditions, and endure the external environment conditions. These functions are to be continually performed until the shelf life period ends. Most of the food and drink containers are subjected to

heat processing in order to prolong the shelf life of the product with the shelf life increasing to 2-3 years for food cans. The processing cycles used are severe and the containers must be specifically designed to withstand conditions of high temperature and pressure cycles in a stream/water environment (Page *et al*., 2003). The heating processes are sterilization; where the cans are made to be free from all microorganisms and pasteurization which is a method of partial sterilization (Abercrombie *et al*., 1990).

Glass containers are also well used in packaging. The American Society for Testing Materials defined glass as “an inorganic product of fusion which has cooled to a rigid state without crystallizing” (ASTM, 1965 in Girling, 2003). Girling (2003) gives the two main types of glass container used in food packaging as bottles, which have narrow necks and jars and pots which have wide opening. Examples of foods packed in glass can be instant coffee, spices, dairy products, sugar just to name a few. The advantages of using glass include transparency, microwavable, ease of opening, heat processable and strength.

In today's packaging efforts, materials like paper and paperboard have also been widely utilised in packaging. In this category, are light weight infusible tissues for tea and coffee bags to heavy duty boards used in transportation. Paper and paperboard packaging is used over a wide range of temperature; from frozen food storage to high temperatures of boiling water and heating in the microwave and convectional radiant heat ovens (Kirwan, 2003). The drawback with the use of paper and paperboard for packaging is its permeability to fluid, and gaseous substances such as water, water vapour, organic solvents, oxygen, and carbon dioxide. However, Kirwan (2003) argues that this form of packaging, can acquire barrier properties and enhance functional performance such as heat sealability for leak-proof packaging, through coating and lamination with plastics such as polyethylene (PE), polypropylene (PP), polyesters (PET) etc., and other wax treatments. These materials can be classified as suited for packaging because of their appearance and performance. Appearance relates to the visual impact of the container and can be expressed in terms of colour, smoothness and a gloss furnish. Performance property

is related to the level of competence achieved during the manufacture of the pack, in printing, cutting and creasing, gluing and the packing operation. Performance property discussed by Kirwan (2003) is also related to pack compression strength in storage, distribution, at the point of sale and in consumer use.

The other material used for the packaging of foods and which will be the focus of this study are organic-macromolecular polymers, simply known as "plastics". Plastics are chemically organic-macromolecular compounds obtained by polymerisation, polycondesation, polyaddition, or any similar process from molecules with a lower molecular weight (monomers) or by chemical modification of natural macromolecular compounds (polymers). The process of polymerisation should be as complete as possible since a typical modern plastic packaging material may contain many different constituents. The constituents either contributes to the safety or quality problems if the material is poorly designed (Brown and Williams, 2003). The plastic packaging material is also known as the polymer or copolymer are manufactured from one or more types of monomers such as styrene, vinyl acetate, ethylene, propylene or acrylonitrile. All polymers contain small amounts of residual monomers left unreacted during the polymerisation process. This then becomes a problem because these constituents are potentially available to migrate into foods (Till *et al*., 1982; Fankhauser-Noti *et al*., 2005).

According to Kirwan and Strawbridge (2003), more than 30 different plastics are used for the packaging of food such as Polyethylene (PE), polypropylene (PP) and polyesters. In these plastics, different types of a group of chemicals known as additives such as antioxidants, stabilizers, lubricants, anti-static and anti-blocking agents, have been developed to improve their performance either during processing and fabrication or in the use of these polymeric packaging material (Lau and Wong, 2000).

Kirwan and Strawbridge (2003) conducted a study on plastics in food packaging where their study focused primarily on the types of plastics, their function and the manufacturing process. There are different properties that these plastics show, which result in their usage in the packaging industry. These include the following:

they are flowable and moldable under certain conditions; they are usually chemically inert, though not necessarily impermeable; they are cost effective in meeting market needs; they are light weight; and they provide choices in relation to transparency, colour, heat sealing, heat resistance and barrier. Most packaging plastics are thermoplastic which means that they can be repeatedly softened and melted when heated. Thermosetting plastics are those which change irriversibly once in contact with heat and pressure (Kirwan and Strawbridge, 2003).

Plastics are utilised in the packaging of food. They offer a wide range of appearance and performance properties. These properties are derived from the inherent features of the individual plastic material and how it is processed and used. They are not highly reactive with inorganic chemicals such as acids, alkalis, and organic solvents. This then makes them suitable i.e. inert for food packaging. A number of plastics may absorb some food constituents, and therefore, it is vital to conduct a thorough testing to inspect all food applications for absorption and migration (Kirwan and Strawbridge, 2003).

1.2 The use of plastics in food packaging

They are used as containers, container components and flexible packaging. In weight, they are the second most widely used and first in terms of value. The usage of plastic in the packaging of food is due to various reasons which include the protection of food from spoilage, the integration of plastics with food processing technology, not interacting with food, being light in weight, are not prone to breakage, do not results in splintering, and are available in a wide range of packaging structures, shapes and designs which then presents food products cost effectively, conveniently and attractively (Kirwan and Strawbridge, 2003).

Plastic films can be used to wrap food and prolong the shelf life of food to about three to nine months. The main function of the plastic films that are used in packaging is to control the internal micro-climate with a range that hinders microbial

competitions. These wrappers are not airtight or hermetic, but they are less porous and hence their choice should be a numerate operation (Oswin, 1982).

1.3 Types of plastics used in food packaging

There are different types of plastics that are used which can be summarised as follows:

Polyethylene (PE), polypropylene (PP), polyesters (PET, PEN, PC), ethylene vinyl acetate (EVA), polyvinyl chloride (PVC) polyamides (PA), polyvinylidene chloride (PVdC), polystyrene (PS), styrene butadiene (SB), acrylonitrile butadiene styrene (ABS), ethylene vinyl alcohol (EVOC), high nitrile polymers (HNP), flouropolymers (PCTEF/PTFE), cellulose-based materials, polyvinyl acetate (PVA), polyethylene terephthalate (PET) (Kirwan and Strawbridge, 2003).

PET is mainly used in soda and water bottles, while PP is used for storage containers, PVC in children's toys, and meat plastic wrappers, while PS is commonly used in fast-food containers and cups (Helmroth *et al*., 2002).

With the use of such packaging materials, there are factors that affect the quality of the packaged foods. This is due to the presence of different molecules or compounds in food packaging materials. This can be predicted at the stage of product development through the composition of the product (intrinsic factors), the environment that the product will encounter during its life (extrinsic factors) and the shelf life limiting processes, in which the combination of both intrinsic and extrinsic factors result. The intrinsic factors include water activity (aw), pH, oxygen availability, and redox potential. The extrinsic factors include time-temperature profile during processing, temperature control during storage and distribution, relative humidity (RH) during storage and distribution etc. (Brown and Williams, 2003).

Hence, the use of appropriate packaging technology to reduce food losses and provide safe and healthy food products is a critical aspect in food packaging. The product quality and shelf life of packaged foods can be affected by unsuitable choice

of packaging material. Packaging can be limiting to the shelf life of the food as a result of migration of tainting compounds from the packaging into the food or migration of food components into the packaging material (Brown and Williams, 2003).

1.4 The use of different packaging material

There are different types of materials used in food packaging. Different types of plastics are used in packaging as discussed in the previous section. Another packaging material used is antimicrobial film. There are two basic types or categories of antimicrobial films. One involves the direct incorporation of the antimicrobial additive into the packaging film, while the second set of the film, is coated with a substance which acts as a carrier for the additive (Cooksey, 2001). In order for this process to be effective, there should be direct contact between the packaging material and the foodstuffs. A new system that is used is vapour-active antimicrobial additives. In this system, there is no direct contact required although it is still crucial that the films formulated in such a way allow controlled release of additives into the foodstuffs. In order for the additives to act against the target bacteria, it is imperative to allow a slow release of the antimicrobial additives. This then helps in the increase of the effect of the antimicrobial action against the target bacteria (Cooksey, 2001).

Figure 1.1 (a) shows how a film with an antimicrobial coating could release the antimicrobial agent onto the surface of the food on the inside of the package. The rate of release would depend on the type of interaction between the antimicrobial agent, the coating material, the targeted bacteria, and the foodstuffs itself.

Figure1.1 (b) shows how an antimicrobial agent could be released from a film which has the antimicrobial agent directly incorporated into the film. This particular diagram suggests that a barrier material might be necessary on the outside layer of the film to prevent loss of the antimicrobial additive to the outside of the package.

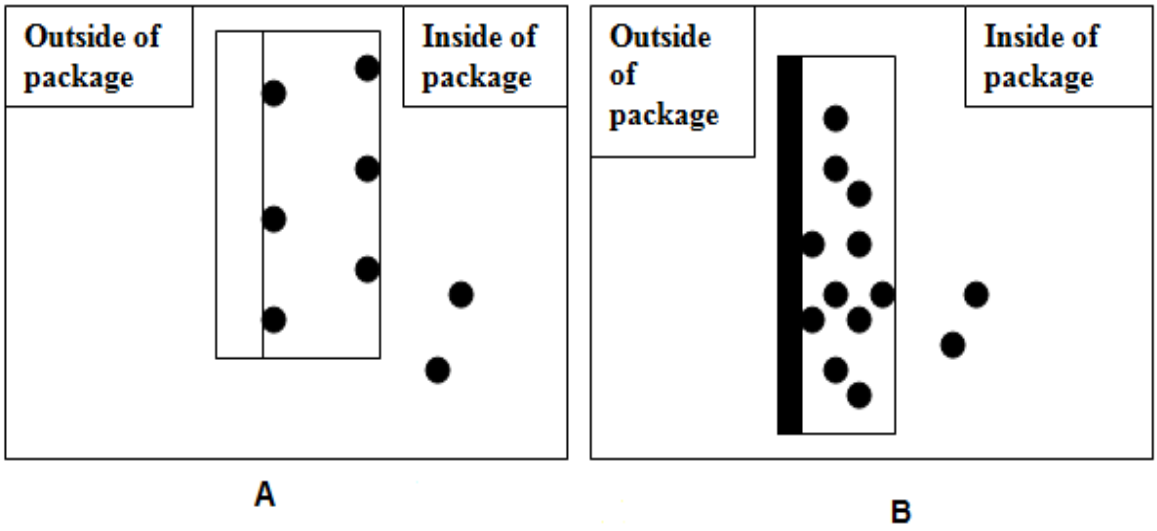

Figure 1.1 The profile of packaging film and polymer film

(Source: Adapted from Cooksey (2001)

a) Profile of a packaging film containing an antimicrobial coating
b) Profile of a polymer film containing antimicrobial agents with a barrier layer on the outside layer of the packaging

Bacteria can become resistance to the antimicrobial agents; it is therefore essential to investigate further the extent to which these bacteria can become resistant. If evidence suggests some level of resistance, then it can safely be concluded that they are not effective. It is vital to remember that most antimicrobial packaging materials should not be used as a substitute for quality sanitation and handling practices. However, these packaging materials, could be used as additional protective measures, to help ensure the safety and high quality of foodstuffs (Padgett *et al*., 1998).

1.5 Phthalates

Phthalate esters (generally referred to as phthalates) are liquids, similar in appearance and consistency to vegetable oils. There are different types of phthalates, each varying in chemical structure and molecular weight (Heudorf *et al*.,

2007). Phthalates are the primary concern of this study. They are used as plasticisers and as additives, in plastics. These compounds are able to migrate and diffuse into foodstuffs which can result in health implications to consumers (Fankhauser-Noti *et al*., 2005a and Fankhauser-Noti *et al*., 2005b). This is due to the direct contact between food and packaging materials which provides the potential for migration. Much work has been carried out into the study of the migration of substances from packaging into food which will be discussed in detail in the literature review.

1.6 Factors affecting migration of phthalates from food contact materials

There are a number of factors affecting the extent of phthates migration from food contact materials into the food. Migration is a process that occurs gradually; hence the duration for which the food and packaging are in contact should be considered (Meiron and Saguy, 2007). During migration, a potential migrating constituent of the packaging is gradually transferred to the food which results in the increase of the concentration of the constituent in the food and a decrease, in packaging material, where a point of equilibrium will be reached. The quality of the constituent in the food at the time of equilibrium is dependent on the physical affinity of the constituent for the packaging and the food (Brown and Williams, 2003).

Balafas *et al*. (1999) also stated that the amount of the phthalate in the packaged foods depends on a number of factors. These include the concentration of phthalate in the packaging material or the printing ink that has been used, how long the food is kept in storage together with the storage temperature, the fat content in the food and the contact area. The higher the phthalate concetration in the packaging material, the higher the migration of the compounds into the food. The migration of the phthalate will also be influenced by the duration of the contact between the food and the packaging material.

The quality, appearance and shelf-life of food can be affected by food interacting with its packaging. Adherence of food residues to the packaging may decrease product acceptability, enhance oxidation and off flavours, increase waste, and result in lowering overall product quality (Michalski *et al*., 1999).

1.7 Regulatory control

In the United Kingdom (UK), the Institute of Food Scince and Technology (IFST) Guidelines (1993) defined shelf life as: *'The period during which the food product will remain safe; be certain to retain desired sensory, chemical, physical and microbiological characteristics; and comply with any label declaration of nutritional data'.*

The consumer always looks for the product with the longest remaining shelf life because it shows a sign of freshness. As a result, organisations such as the EU Directive on food labelling (79/112/EEC) requires pre-packaged foods to have a best before or a use by date. The legislation does indirectly impact the shelf life of food, for example, in a case where different additives will be added to the food in order to increase its shelf life, the legislation relating to the use of additives; those permitted for use and the permitted levels; is relevant. There is also legislation regarding food contact materials which ensures that no components of food contact materials which are likely to endanger health or food quality are transferred into foods. In the UK, there are requirements for food contact materials that are represented by two food regulations under the Food Safety Act. 'The Materials & Articles in Contact with Food Regulations 1987 Statutory Instrumnet No. 1523, as amended by Statutory Instrumnet 1994 No. 979', originated from EC Directive 76/893/ECC, includes the requirements that:

"Materials and particles must be manufactured in compliance with good manufacturing practice so that under their normal or foreseeable conditions of use, they do not transfer their constituents to foodstuffs in amounts that could: endanger human health, or bring an unacceptable change in the composition of the foodstuffs or deterioration in the organoleptic characteristics thereof".

These limits are mainly prescribed for the quantity of vinyl chloride monomer which may be transferred to food, with regards to the materials and articles manufactured with polyvinyl chloride(PVC) such as plastics, which are the main focus of this research. 'The Plastic Materials and Articles in Contact with Food Regulations 1998 Statutory Instrumnet No. 1376, as amended by Statutory Instrumnet 2000 No. 3162'

prescribes limits in relation to the contents of materials and articles intended for food contact and the migration of constituents into food. The methods that can be used in testing of the migration into foods are also defined.

The Council Directive 82/711/EEC proposes basic rules necessary for measuring the migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. The Council Directive 85/572/EEC specifies a list of food stimulants to be used for resting migration from plastic food contact materials and articles. The Commission Directive 90/128/EEC is a key directive for plastic materials intended for food contact. These three directives form part of the European Council and Commission Directives which are implemented by the Regulations discussed above.

Grob *et al*. (2007) stated that the European legislation regulates migration from food contact materials, such as packaging, into foods by an overall migration limit (OML) applicable to the total of the migrating material and specific migration limits (SMLs) referring to individual substances or groups of substances. From the European legislation, a general OML of 10mg/dm^2 contact area sets a limit on the maximum quantity of constituents allowed to transfer out of plastic materials and articles into the food (Grob *et al*., 2007). The Directive led to the establishment of approved monomers and starting substances which are permitted for use in food contact plastics. There are also specific migration limits (SML) that are set for some monomers and starting substances (Grob *et al*., 2007).

Consumers need to be protected from the migration of harmful substances from packaging of foodstuffs. Lau and Wong (2000) stated in their review that the Commission of the European Communities (CEC) Directive is implemented for plastics of packaging materials within the European communities. Since 1976, the CEC Directive introduced limits upon the overall migration of harmful substances from plastics into food and food stimulants. An OML of 10 µg/dm^2 or 60 mg/kg of food stimulant was set which also includes lists of permitted monomers together with

the restrictions which apply to specific monomers (CEC Directive 90/ 128/EEC, 1990).

1.8 Food safety

Food safety is the component of environmental health that protects food supply and involves a collaboration between the food industry and regulatory agencies internationally and locally. The main goal of this collaboration would be to enhance safety of food supply and reduce the incidence of foodborne illness. Foodborne illness is the sickness that people experience after consuming food and beverage that is contaminated with pathogenic (disease-causing) microorganisms, chemicals or physical agents (McSwane *et al*., 2005).

There are many incidences or outbreaks of foodborne illness that have occurred over the years. The estimates of these outbreaks vary annually. In 1993, the U.S. FDA estimated an annual burden of between 24 and 81 million cases of foodborne illness, which resulted in, an estimated number of 10,000 deaths (FDA, 1993). This underpins the extent and severity of potential outbreaks and why it is imperative to conduct researches that can lead to the reduction of such occurrences.

Food contamination occurs at many points as it flows from harvest through processing and distribution to the consumer. Hence, it then becomes important to look thoroughly at the sources of food contamination. Figure 1.2 shows the different common sources of food contamination that contribute to the cause of foodborne illness. Based on the evidence gathered, it would be resoanable to conclude that food can be sterile during preparation stage, and be exposed to contamination at the packaging and distribution stages, or through exposure to air.

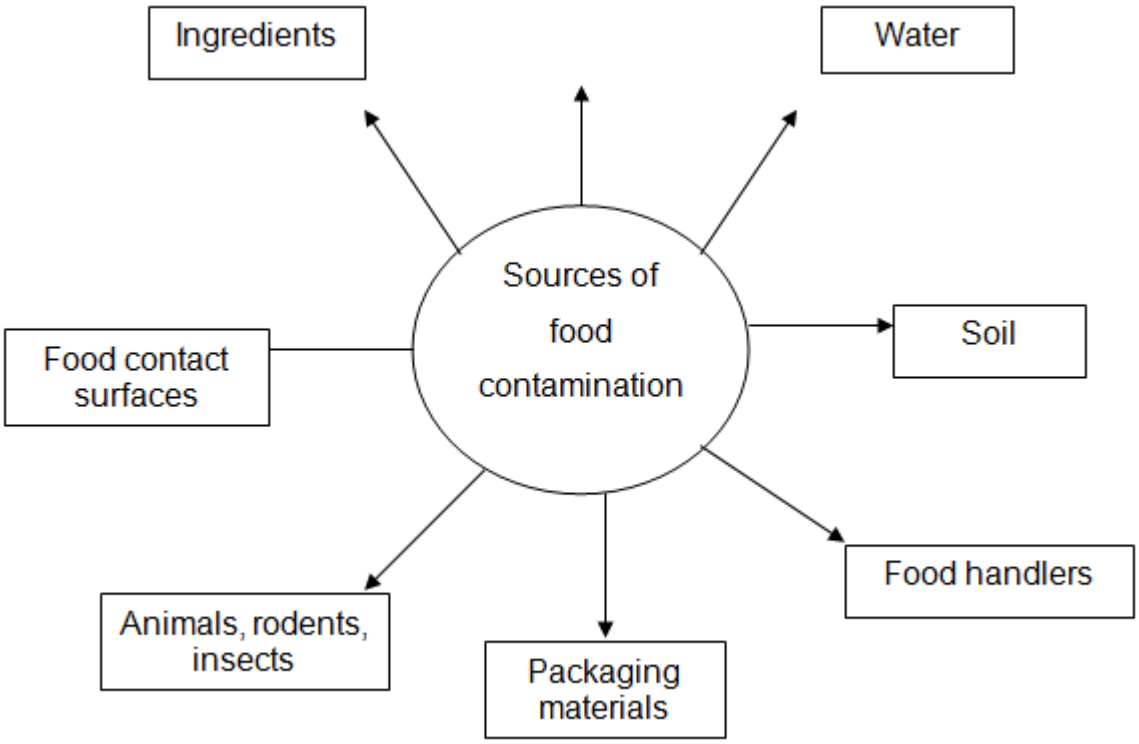

Figure 1.2 Common sources of food contamination

Source: (Oswin, 1982)

The packaging of perishable foods is a form of transfer engineering that is provided to assemble contents and prolong their shelf-life in a hostile environment. For many foodstuffs, a shelf-life of a few weeks or months is adequate, and only flexible plastic food wrappers meet this need. These plastic wrappers impede oxygen and water vapour from the surrounding micro-environment or climate, as a result, they retard the activity of microbes, and reactions which spoil flavour (Oswin, 1982). There are different modes of packaging used such as active packaging which involves the package, the product and the environment interacting to prolong shelf life or enhance safety while maintaining the quality of the product (Suppakul *et al*., 2003). The use of the different packaging methods depends on the type of food that is being packaged, for example, for meat, plastic packaging is used and for other foodstuff such as sugar paper or plastic packaging can be used.

1.9 Food pathogens and their health implications

In food, both good and bad bacteria are found (Wassenaar, 2005). The good bacteria are those that are found in foodstuffs such as dairy products, eg., *Lactobacillus lactis* in cheese. However, there are also bad bacteria that can lead to the spoilage of food or even illness to humans such as *Escherichia coli O157, Bacillus cereus* and many more.

There are different food pathogens found in various food which are responsible for the spoilage of food. These bacteria can cause foodborne illness in humans. Foodborne illnesses develop because of the consumption of food or drinking beverages contaminated with bacteria, parasites or viruses although harmful bacteria are still said to be the most common causes. Some of the bacteria can be present in food when purchased but they are commonly found in raw foods such as raw meat and poultry since they are not sterile and may have been contaminated during slaughter (NIH, 2007). The most common food pathogens are *Bacillus cereus; Staphylococcus aureus; Salmonella spp.; Escherichia coli O157; Listeria monocytogenes; Campylobacter jejuni;* and *Clostridium perfringens*. which can be found in different foods.

The pathogenicity of these food pathogens depends on the strain of a particular bacterium. For example, *Escherichia coli* (*E. coli*) has multiple strains. Some of these strains are not regarded as pathogens, however, they can be opportunistic pathogens that cause infections in immuno-compromised hosts (Neill *et al*., 1994). Therefore, the evaluation of the presence of these bacteria in the food samples under study is imperative since these food samples are consumed in many households on a daily basis.

1.10 Problem statement

It has been established that there are different types of plastic wrappers which are used in food packaging. The study is focused on plastic packaged food materials that are sold in commercial stores to consumers. The plastic materials contain additives such as plasticisers, whose addition or presence is to ensure the flexibility of the plastic material during production. Plasticisers are chiefly used to turn PVC from a hard plastic into a flexible plastic (Wang, 2000). An example of such

platicisers are phthalate esters, some of which has been found in food substances and are highly detrimental to human health at certain level.

The study focuses on evaluating the presence of these compounds in the plastic-food wrappers and the levels to which they exist in different food samples in comparison with the acceptable limits as set by standards of European Commision and the United States Environmental Protection Agency . Literature on the presence and migration of phthalate esters in foods has been reported mostly in developed countries such as US and UK, however, there is scarcity of information in developing countries such as South Africa, hence importance of this research work. Plastic packaged food substances are purchased from commercial stores and consumed by people on a daily basis and such packaged foods include cheese, polony, and vienna. The cumulative effects and health implications of the presence of these toxic compounds in humans through the consumption of phthalates esters ladden foods is of great concern, hence the need to conduct this study.

1.11 Justification of study

In South Africa, the literature review process revealed that no study has been conducted to investigate the presence of phthalate esters in plastic food wrappers and different foods that are commonly found in departmental stores wrapped in these plastic wrappers. Most studies have been conducted in the United States and Europe and have focused mainly on the presence of these compounds in the plastic wrappers, their migration into foodstuffs and the health implications thereof (Fankhauser-Noti and Grob, 2006; Goulas *et al*., 2007). This study adds a new dimension to previous investigations, as it focuses on the likely impact of the presence of these compounds in the plastic food wrappers that are used to wrap foodstuffs which are commonly used in many households, in South Africa. Polony, cheese and vienna were selected for this study. In different retail stores, these food samples are usually wrapped in flexible PVC films; as shown in figure 1.3 below; and kept refrigerated in order to retain their characteristic properties.

Cheese **Polony** **Vienna**

Figure 1.3 Pictorial presentation of the food samples wrapped in plastic

Some health problems in the society might be due to the presence of phthalates compounds as a result of consumption of foods ladden with phthalates. If ways to minimise this problem are not found, then extreme health implications will continuously be experienced in both children and adults. Much study has been conducted in order to evaluate the effect of phthalate exposure to both children and adults. However, much of this study was done on rodents due to lack of human testing. In a study on maternal phthalate exposures and health outcomes, Swan *et al*. (2005) found a relationship between increased maternal phthalate concentrations in pregnancy and decreased anogenital distance. Although it has been found that exposure to phthalates does not result in bioaccumulation because of their low molecular weight (Heudorf *et al*., 2007), more potential detrimental health effects such as developmetal and reproductive toxicity of phthalates is of great concern..

1.12 Aim and objectives of the research work

The main aim of this research project was to investigate the possible prevalence of some chemical compounds (phthalate esters) and microbiolgical entities in food samples from selected commercial stores in Tshwane Metropolitan Area of Gauteng Province in South Africa.

In order to achieve this aim, the following objectives were targeted:

1. To investigate possible prevalence of phthalate esters DEHP, DMP, DnBP, DEHA, and BBP in plastic wrapped foods from selected commercial stores in Tshwane Metropolitan Area of the Gauteng Province in South Africa.
2. To examine possible incidences of microorganisms such as *Salmonella,* coliforms, yeast and mould, *E. coli, Streptococcus, Listeria monocytogenes* and *Clostridium botulinum* in these foods.
3. To compare the level of these chemico-microbial entities if detected to standards.
4. To unravel possible health implications to consumers if the above mentioned compounds and microorganisms are present in food sampled.

1.13 Research Hypothesis

1. There is high probability of the presence of phthalates esters in foods that are wrapped or packaged in plastics which contain phthalates esters.
2. There is the presence of microbial compounds in some food samples especially polony in view of the form of packaging.

1.14 Purpose of study

The purpose of this research is to determine the presence of phthalates in selected food samples and plastic food wrappers. The compounds suspected to be in the plastic wrappers will be assessed in the food samples and the levels at which they are found will be compared to the acceptable, legal limits. The presence of any pathogens will be determined using different microbiological methods.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Different studies have been conducted on the safety of plastic-food wrappers, their chemical content and the development of microbes in food; which led to, the review of the legal limits on the migration of hazardous chemicals into foodstuffs. This chapter covers the arguments and findings of other researchers who have worked with phthalate esters (Heudorf *et al*., 2007), their effects and how they migrate from the plastic wrappers into food (Page and Lacroix, 1995; Bonini *et al*., 2008 and Goulas and Kontominas, 1996). The various methods used in determining the presence of phthalates esters are examined.

2.1.1 Phthalates

2.1.1.1 Background

Phthalate esters, diesters of 1,2-dicarboxylic acid, are a group of low volatility, water insoluble, highly lipophilic (fat soluble) organic chemicals (Hauser *et al.*, 2004a, Green *et al*., 2005, Lee and Veeramachaneni, 2005; all cited in Wong, 2008). As stated in section 1.5 and 1.6, phthalate esters are found in a wide range of consumer and food packaging products, which then leads to an extensive exposure of the human population to these chemicals. Figure 2.1 shows a general structure of phthalates.

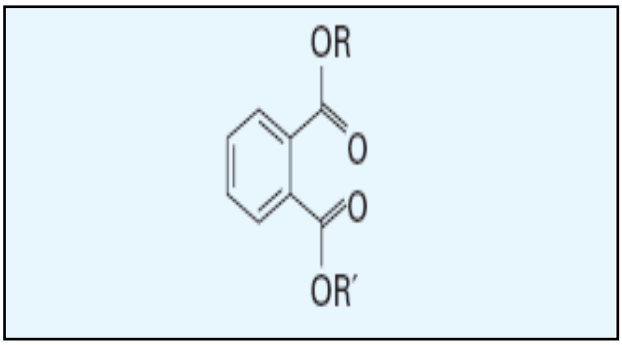

Figure 2.1 General chemical structure of phthalates

Source: Calafat and McKee (2006)

Anderson (2001) found that the phthalate diesters are rapidly converted to their monoesters in humans and in rats (Rowland, 1977) and according to Foster (2006), the monoester metabolite is believed to be the toxic chemical. Several phthalates and their metabolic products were shown to be developmental and reproductive toxicants, which mostly have an impact on male reproductive development (Gray *et al*., 2000, Ema and Miyawaki, 2002).

2.1.1.2 Identity and properties

Plasticisers are widely used in polymeric units, to alter and modify their properties. Much research has been done on these chemicals, in order to understand their function and the implications that their use might have (Fankhauser-Noti and Grob, 2006; Goulas *et al*., 2000). The International Union of Pure and Applied Chemistry (IUPAC) describes plasticisers as low molecular weight synthetic organic esters of high-boiling points incorporated in a material, to increase its flexibility, processibility and extensibility (Giam and Wong, 1987; Brody and Marsh, 1997). Wong (2000) added by stating that a plasticiser may reduce the melt viscosity, lower the temperature of a second-order transition or lower the elastic modulus of the product. Most plasticizers are liquids of low volatility. In many circumstances, they are blended to produce a wide range of physical properties from a single parent polymer (Wang, 2000). Since plasticisers are used to alter or modify the properties of a polymeric system, it is essential to consider its compatibility, permanance, aging and its effect on the other properties (Deanin, 1978). Table 2.1 below shows name and structural formula different plasticisers.

Table 2.1 Name and structural formula of different plasticizers

Name	Acronym	Structural formula	CAS No.
Dimethyl phthalate	DMP	$C_6H_4(COOCH_3)_2$	131-11-3
Diethyl phthalate	DEP	$C_6H_4(COOC_2H_5)_2$	84-66-2
Diallyl phthalate	DAP	$C_6H_4(COOCH_2CH=CH_2)_2$	131-17-9
Di-n-propyl phthalate	DPP	$C_6H_4[COO(CH_2)_2CH_3]_2$	131-16-8
Di-n-butyl phthalate	DBP	$C_6H_4[COO(CH_2)_3CH_3]_2$	84-74-2
Diisobutyl phthalate	DIBP	$C_6H_4[COOCH_2CH(CH_3)_2]_2$	84-69-5
Butyl cyclohexyl phthalate	BCP	$CH_3(CH_2)_3OOCC_6H_4COOC_6H_{11}$	84-64-0
Di-n-pentyl phthalate	DNPP	$C_6H_4[COO(CH_2)_4CH_3]_2$	131-18-0
Dicyclohexyl phthalate	DCP	$C_6H_4[COOC_6H_{11}]_2$	84-61-7
Butyl benzyl phthalate	BBP	$CH_3(CH_2)_3OOCC_6H_4COOCH_2C_6H_5$	85-68-7
Di-n-hexyl phthalate	DNHP	$C_6H_4[COO(CH_2)_5CH_3]_2$	84-75-3
Diisohexyl phthalate	DIHxP	$C_6H_4[COO(CH_2)_3CH(CH_3)_2]_2$	146-50-9
Butyl decyl phthalate	BDP	$CH_3(CH_2)_3OOCC_6H_4COO(CH_2)_9CH_3$	89-19-0
Diisoheptyl phthalate	DIHpP	$C_6H_4[COO(CH_2)_4CH(CH_3)_2]_2$	41451-28-9
Di(2-ethylhexyl) phthalate	DEHP, DOP	$C_6H_4[COOCH_2CH(C_2H_5)(CH_2)_3CH_3]_2$	117-81-7
Di(n-octyl) phthalate	DNOP	$C_6H_4[COO(CH_2)_7CH_3]_2$	117-84-0
Diisooctyl phthalate	DIOP	$C_6H_4[COO(CH_2)_5CH(CH_3)_2]_2$	27554-26-3
n-Octyl n-decyl phthalate	ODP	$CH_3(CH_2)_7OOCC_6H_4COO(CH_2)_9CH_3$	119-07-3
Diisononyl phthalate	DINP	$C_6H_4[COO(CH_2)_6CH(CH_3)_2]_2$	28553-12-0
Diisodecyl phthalate	DIDP	$C_6H_4[COO(CH_2)_7CH(CH_3)_2]_2$	26761-40-0
Diundecyl phthalate	DUP	$C_6H_4[COO(CH_2)_{10}CH_3]_2$	3648-20-2
Diisoundecyl phthalate	DIUP	$C_6H_4[COO(CH_2)_8CH(CH_3)_2]_2$	85507-79-5
Ditridecyl phthalate	DTDP	$C_6H_4[COO(CH_2)_{12}CH_3]_2$	119-06-2
Diisotridecyl phthalate	DIUP	$C_6H_4[COO(CH_2)_{10}CH(CH_3)_2]_2$	68515-47-9

Source: Adapted from Wikipedia, 2008

Wang (2000) conducted a study on the polymer additive using pyrolysis gas chromatography with the main focus on plasticisers. It as highlighted in the study that a plasticiser must be capable of being mixed uniformly and homogeneously and remain blended when cooled to room temperature and throughout the useful life of the plastic product. This means that the stability of the plastic in its mixed state must be maintained throughout its useful life.

2.1.1.3 Uses

Many compounds can be used as plasticisers. These include phthalates, adipates, phosphates, citrates and sebacates. Phthalates are added in different personal-care products (e.g. perfumes, lotions, and cosmetics), certain medical devices, pharmaceuticals, paints, and industrial plastics. Plasticisers can be used as lubricants, citrates, flame retardants or thermal stabilizers (Wang, 2000).

Table 2.2, gives a summary of the different phthalates and their uses. Through this, it is observed that these phthalates might be harmful but they also have a positive applications in different industries.

Table 2.2 The use of phthalates as plasticisers

Phthalate	Use
DEP	Personal care products, cosmetics
BBP	Vinyl tiles; food conveyor belts, artificial leather, automotive trim, traffic cones
DBP	PVC plastics, latex adhesives, cosmetics, personal care products, cellulose plastics, solvent for dyes
DEHP	Building products (wallpaper, wire and cable insulation), car products (vinyl upholstery, car seats), clothing (footwear, raincoats), food packaging, children's products (toys, grip bumpers), medical devices
DnHP	Dip-moulded products, such as tool handles, dish-washer baskets; flooring, vinyl gloves, flea collars, conveyer belts used in food processing
DnOP	In mixtures C6–C10 phthalates: garden hoses, pool liners, flooring tiles, tarps Seam cements, bottle cap liners, conveyor belts (indirect food additive!)
DINP	Garden hoses, pool liners, flooring tiles, tarps, toys
DIDP	PVC plastics, covering on wires and cables, artificial leather, toys, carpet backing, pool

Source: Adapted from (Heudorf *et al.,*2007) and Benson (2009)

2.2 Additives in food

Additives are used to aid the production of polymers and to amend the physical properties of the finished material. The Antioxidants, stabilizers and plasticizers, can be classified as plastic additives. These additives have a significant influence in the processing and shelf-life of plastics and are responsible for many of the properties of

these materials. Haider and Karlsson (1999) conducted a study on additives, focusing on a rapid ultrasonic extraction technique used to identify and quantify additives in polyethylene. The effect of temperature, time and structure of the additives on the extraction efficiency showed that the recovery of the additives increased extraordinarily with higher temperature and longer time.

Polyvinyl chloride (PVC) is one of the most versatile plastics because of its blending ability with a variety of additives such as plasticizers and stabilizers to produce a wide range of products including packaging materials (Goulas *et al*., 2000). About 25% of PVC in packaging involves food applications, 31% medical uses, and 41% nonfood uses. In food packaging, PVC applications are largest in film, sheet and bottle production (Brody and Marsh, 1997).

In this research project, the main focus is the presence of chemicals in food packaging materials. There are many different sources of migrating constituents that can migrate into foods. From the packaging of each foodstuff, adhesives used to seal packaging can also be one of the sources of migrating constituents. Adhesives commonly used are hot melt, pressure sensitive, cold seal, water-based, solvent-based, solvent-free, acrylics and poly-urethanes (Brown and Williams, 2003). Bonell and Lawson (1999) compiled a list of substances used in the manufacturing of adhesives for food, from a survey of adhesives manufacturers. More than 360 adhesives were found to be commonly used.

2.3 Plasticisers in packaging films

Phthalates are used as plasticisers in PVC plastics. There are different types of phthalates that are widely used; namely DEHP, di-iso-nonylphthalate (DINP), di-iso-decylphthalate (DIDP), di-ethyl-phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP) and di-n-octyl phthalate (DnOP).

The thin PVC films also known as cling films are widely used for food; wrapping red meat, poultry, fruits, vegetables, and cheese at retail level. PVC film is widely used as a food wrap because it is flexible and transparent and has a relatively high permeability to oxygen and water (Davis, 1996). These plasticizers have been reported to elute constantly at a specific rate from plastic products to the

environment (Latini, 2005) which then lead to them being widely distributed in the ecosystem.

Plasticisers can be classified in different ways; such as molecular mass, molecular structure, compatibility, cost efficiency or purpose of application. Analytically, they can be classified by their molecular structure because it is directly related to polarity and molecular flexibility which will affect their properties (Beeler and Finney, 1979-1980).

Plasticisers such as phthalates, adipates, phosphates, citrates and sebacates are widely used in food packaging materials (Van Lierop and Van Veen, 1998), and they are usually added in significant amounts. Most of these plastic films used to package foods are plasticised with high levels of DEHA (Sandberg and Vaz, 1984 cited in Goulas *et al*., 2000), which also happens to be the most widely used plasticiser for food contact applications in PVC. DEHA is often used in concentrations exceeding 20% by weight of the polymer. Oswin (1981) discussed the different plastic wrappers used in the different types of foods. It was shown that plastic film used in packaging bread is different from that which is used to package vegetables, red meat or snack foods. When choosing the appropriate wrapper, there are factors which affect food packaged; especially in plastic wrappers. These factors were discussed by Oswin (1981). They include permeability of oxygen, water activity, water vapour, and storage atmosphere. In order to prolong shelf life flexible wrappers should impede oxygen and water vapour from the contained microclimate which will result in the retarding of microbial activity. Thus, food packaging forms a significant part of this research as it is the core of the contamination of the selected food samples which will be studied

2.4 Migration of plasticisers into food

Phthalates plasticisers are not chemically bound to PVC; they can leach, migrate or evaporate into indoor air and atmosphere, foodstuff and other materials. As a result, the use of plasticized PVC as cling film has been targeted as a potential problem in terms of migration. Consumer products containing phthalates can result in human exposure through direct or indirect contact. Indirect contact can be in the form of

inhalation, ingestion and skin exposure (Heudorf *et al*.,2007), although this will be discussed further in this chapter.

The wide use of phthalates in various products and their ability to migrate into various environmental compartments has encouraged the detection of phthalates not only in consumer products, but also in food. Although several phthalates have been approved as indirect additives i.e. as adhesives and components of food wrapping (Goulas *et al*., 1998), it was reported by Page and Lacroix (1995) that phthalate levels are quite variable, and packing materials are regarded as a relevant emission source. This shows that there are different ways in which phthalates are released into different foods. For example, in the study conducted by Petersen and Breindahl (2000) it was found that some disposable PVC gloves used during the preparation of meals were suspected as one of the sources of high DEHP content. To confirm this fact, Tsumura *et al*. (2001) studied the contamination of retail foods caused by PVC gloves used in the preparation. Sixteen packed lunches and ten set lunches were analysed for DEHP using GC/MS. The concentration of DEHP was found to be the highest in all samples; i.e. 0.8-11.8 mg/kg in packed lunches and 0.012-0.3 mg/kg in set lunches. DEHP content of five packed lunches exceeded 1.85mg (EU TDI) for 50kg body weight. PVC gloves contained 22 or 41% weight of DEHP and were sprayed with alcohol before used for sterilization. It was concluded in this study that the alcohol increased the migration of DEHP into the food.

Food that is wrapped in plastic containing plasticisers was studied by Bonini *et al.* (2008) in order to evaluate the migration of plasticisers into the foodstuffs. In this study, it was found that the migration of compounds such as DEHA reached too high values. It was then recommended that the use of polymeric plasticisers instead of the migration monomeric plasticisers will reduce the amounts that migrate into the food. It was also ascertained that migrated quantities do not depend on the time of contact; such then, foods wrapped for quite a long time did not represent a risk, for the consumers, of an increased intake of plasticisers. This then indicated that prolonged exposure of the foodstuff to plasticiser containing wrapper, does not increase the amount of plasticisers that will migrate into the food. The amount of the migrant solely depends on the amount of plasticiser present in the polymer.

However, this finding has been argued by many researchers and it will be further dicussed in chapter 5.

Sweetened sesame paste (halawa tehineh), also known as halva, is a common sweet food in Greece and the Middle East. It contains sugar, glucose, vegetable oils and tahina. Goulas *et al*. (2007) investigated the migration of DEHA and acetyltributyl citrate (ATBC) plasticisers from PVC film into this common food. Kinetic and penetration studies were conducted using a direct gas chromatography (GC) method after extraction of DEHA from the samples. It was found that DEHA readily leached into halva samples. The equilibrium amount of DEHA in halva was 81.4 mg/kg, which was corresponding to a loss of 54.7% (w/w) DEHA from PVC film. The concentration 81.4 mg/kg was compared to the limit of 3 mg/dm^2 of film surface set by the EU for DEHA and was found to be slightly higher. With ATBC, the equilibrium amount in halva was 36.1 mg/kg, which was corresponding to a loss of 42.7% ATBC from PVC film (Goulas *et al*., 2000).

With regard to the penetration of both plasticisers into halva samples, migration of DEHA was detectable up to the 7th slice beneath the surface of halva (total depth 10.5 mm) while the migration of ATBC was detectable up to the 5th slice (total depth 7.5 mm). This gives more evidence that plasticisers can migrate into food. It was concluded that although the PVC film studied contained a polymeric (low migration) plasticiser and significantly lower amounts of monomeric plasticisers than conventional PVC films, relatively high levels of DEHA plasticizer migrated into the product (slightly higher than the EU imposed limit of 3mg/dm^2 film). This was due to high fat content in halva (sweetened sesame paste).

Phthalates are able to migrate into the food due to the contact time. Brown and William (2003) discussed the studies on migration of the plasticiser DEHA conducted by Startin *et al.* (1987) and Castle *et al.* (1987). This plasticiser was found to be migrating into the food during home-use and microwave cooking also in retail-food packaging. It was stated that the level of migration increased with the length of exposure time and temperature of exposure. The highest levels were found where there was a direct contact between the film and the food. Castle *et al*. (1988)

suggested the use of a thinner film as a means of reducing the migration of this plasticiser.

Various studies have been done on the migration of plasticisers into cheese. An example of this is the study conducted by Goulas *et al.* (2000). PVC film containing DEHA was used to wrap different types of cheese; i.e. Kefalotyri, Edam, and Feta. Kinetic and penetration studies were conducted after 240h of contact. Statistically significant differences in migration of DEHA were observed between the cheese types. Migration of DEHA depended on contact time, fat, and moisture contents, and consistency of cheese samples. After 240h of contact under refrigeration, the migration of DEHA was approximately 345.4mg/kg (18.9mg/dm^2) for Kefalotyri, 222.5mg/kg (12.2mg/dm^2) for Edam, and 133.9mg/kg (7.3mg/dm^2) for Feta. The loss of DEHA from the PVC film into the three cheese types was 37.8, 24.3, and 14.6%, respectively. These values, with the exception of feta, were higher than the upper limit for global migration from plastic packaging materials into food and food stimulants set by the EU (10mg/dm^2 or 60mg/kg). It was then concluded that high levels of DEHA are found in all three cheese samples tested. From the results, it was observed that migration values exceeded in all cases the upper limit for DEHA migration proposed by the EU (18mg/kg). Furthermore, DEHA migration exceeded the upper limit for global migration set by the EU (10mg/dm^2) with the exception of Feta cheese. The effect of the fat content on the migration of the plasticiser was clearly illustrated.

Goulas and Kontominas (1996) concluded that plasticisers migrate into meat. In their research article, food-grade PVC film containing 28.3 % dioctyladipate (DOA) plasticizer was used to wrap chicken meat samples, with and without skin, contained in a polystyrene tray. The samples were irradiated with γ-radiation [60Co] at doses equivalent to 4 kGγ and 9 kGγ corresponding to cold pasteurisation. Analysis for DOA at intervals between 7hr and 240hr of contact to contaminated chicken samples was done. An indirect GC method was used for the analysis. Similarly, identical non-irradiated (control) samples were analysed for their DOA content. Results showed no statistically significant differences in migrated amounts of DOA between irradiated and non-irradiated samples. No differences were observed between samples irradiated at 4 kGy and 9 kGy. After 240hr of sample-film contact under

refrigeration, loss of DOA was approximately 35.6 % for chicken flesh plus skin samples and 14.3% for chicken flesh samples.

It has also been shown that mineral hydrocarbons such as liquid paraffin, white oil, petroleum jelly, hard paraffin and microcrystalline wax, have been used in certain polymers as processing aids (Castle *et al*., 1991; Castle *et al*., 1993a; Castle *et al.,* 1993b). Castle *et al*. (1991) detected them in PS containers at levels between 0.3 and 3%. In polystyrene and ABS pots and tubs, they were found in levels between 0.3 and 5.5% (Castle *et al.,* 1993a) and in cheese coatings, they were found at levels up to 150mg/kg^{-1} (Castle *et al.,* 1993b). The migration of these compounds was also detected in food in the same study and this was due to the temperature and fat content.

In a study conducted by Carlo *et al.* (2008), it was stated that a potential risk of phthalates contamination during winemaking exists due to their widespread use, environmental persistence, abundant presence in various plastic materials (including packaging, pumps, tubing). It was reported that this contamination could have been caused by the use of plastics during processing; also from the grapes as a result of their exposure to phthalates from the environment. However, Carlo *et al.* (2008) also highlighted that there is not any report, on the detection of phthalates in grape wines.

2.5 Endocrine Disrupting Compounds (EDC's)

2.5.1 Background

Phthalates are classified as endocrine disruptors. The Environmental Protection Agency (EPA) defines endocrine-disrupting chemicals (EDCs) as, "Exogenous agents that interfere with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance, homeostasis, reproduction, development and/or behaviour (Crisp, *et al*., 1997)." Exogenous substances or mixtures, that possess properties, which might be expected to cause an imbalance within the endocrine system; by altering the function of the endocrine system and hence causing adverse health effects in an intact organism, its progeny, or populations can be classified as EDCs. This term

endocrine disruption has become associated with “falling sperm counts” and “environmental oestrogens” (Fisher, 2004).

In Colborn *et al*. (1993), it was stated that endocrine disrupting compounds disrupt hormones and other components of the endocrine system, which coordinates both the development and the functioning of all organ systems in the adult body. Hence, the disruption of hormones can affect the function of many organs in the body. The endocrine system is not only concerned with the reproductive tract, but with all the hormone-producing organs/glands, which maintain bodily homeostasis. These include the thyroid, parathyroids, anterior and posterior pituitary, pancreas, adrenals, pineal, and the gonads, as presented in Figure 2.2 below (IPCS, 2002 in Fisher, 2004). Exposure to phthalates can disrupt the functioning of the organs illustrated in the pictorial presentation of the endocrine system. It is then important in this study to explore different ways to minimise the leaching of phthalate esters into food. With focus on Figure 2.2, hormones produced by one organ can affect the function of other systems. For example, the central gland of the endocrine system, the hypothalamus is key to the nervous system interacting with the endocrine system. This then shows that the disruption of one hormone or gland could have an effect on the entire endocrine system.

Examples of EDCs include pharmaceuticals and personal care products, general anthropogenic compounds, pesticides, biogenic compounds and inorganic and organometallic compounds (MPCA, 2008).

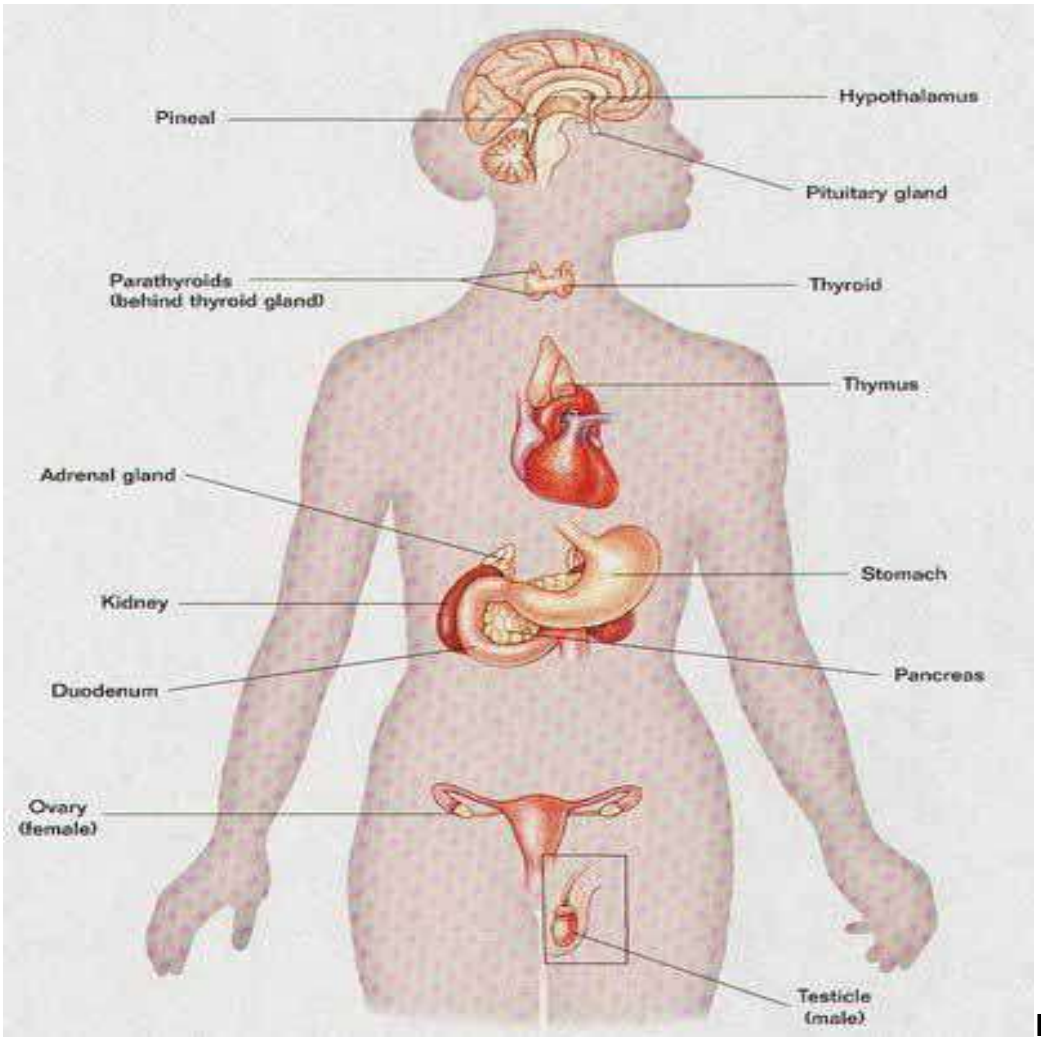

Figure 2.2 Pictorial presentation of the human endocrine system (Adapted from www.epa.gov/endo/images/endocrine.jpg; Accessed 24 March 2010)

2.5.2 Mechanisms of action

There has been greatest concern associated with EDCs. EDCs alter by mimicking, antagonizing, blocking or triggering the actions of naturally occurring hormones and disrupt normal body development, function and activity. In other words, they can directly bind to, or block, hormone receptors, thereby initiating or blocking receptor-activated gene transcription (Amaral, 2002). Solomon and Schettler (2000) stated that other exogenous chemicals act indirectly on hormonal homeostasis by altering steroidogenesis, hormone transport on binding proteins, receptor numbers on target organs or hormone metabolism. An example of this is when the polychlorinated biphenyls (PCBs) interfere with thyroid function by interfering with T4 delivery to the developing brain by displacement from the carrier protein, and interference with the conversion of T4 to T3 (triiodothyronine)(Brouwer *et al*., 1998).

One mode of action of toxic chemicals is that once they have entered the human body, internal toxic effect occurs. Wong (2008) explained that the chemicals then enter the bloodstream by passing through absorption membrane barriers. Barr *et al*. (2005) elaborated; stating that once absorption of the toxic chemical into the bloodstream occurs, the chemical is distributed to primary deposition sites. In these sites, equilibrium is constantly maintained between levels of the chemical in the sites and in blood. Maintenance of equilibrium leads to concentrations of the chemical being slowly released from the storage site as it is eliminated from the blood, and may reach target organs (Barr *et al.,* 2005). When the chemicals have reached target organs, they are metabolised in the liver leading to the reduction of toxicity and hence becoming more hydrophilic (easily absorbed or dissolved in water). Elimination of these metabolised chemicals then occurs via urine, faeces, exhalation; followed by being deposited in secretory structures and then excreted as tears, saliva, sweat or breast milk; as well as discharged through a pregnant woman's bloodstream via the placenta and into the foetal blood supply (Barr *et al*., 2005).

According to Amaral (2002), chemicals can potentially act on the endocrine system in a variety of ways; EDCs can function as estrogens, antiestrogens, antiandrogens, steroidogenic enzyme inhibitors and their receptors. It was highlighted in Boerjan *et al.* (2002) that these EDCs can interfere with reproductive function by binding to the oestrogen (mimic female hormones) receptor, androgen (mimic male hormones) receptor, or plasma binding proteins, which regulate the biological activity of the endogenous steroids.

2.5.3 Exposure to EDCs

2.5.3.1 Sources

Humans are exposed to EDCs in various ways such as through inhalation of contaminated air, ingestion of contaminated food, and contact with contaminated fluids, which can lead to, the absorption of EDCs through the skin (Figure 2.3 and 2.4).

Another source of exposure is in foetuses in the womb, which may be exposed to the toxic chemicals due to the pollutants accumulation that occurred in the bodies of the mothers. Schuiling and van der Naad (2005) widely explored the impact of this

exposure to the foetus and early development in children. Toxic pollutant in the foetus can have serious and dangerous health consequence in adulthood and even throughout generations. For example, Duty *et al.* (2004; 2003a; 2003b) described that there is an altered sperm and semen quality in males after exposure of EDCs. Another example is Sharpe (2005) in an extensive study on the male reproductive health disorders and testis development. It was stated that abnormal development of the testis in foetal or neonatal life can have life-time consequences on all aspects of reproductive function in adulthood, including sperm counts.

The environment can also be classified as another source through manufacturing, use, recycling and disposal (Wong, 2008). Boerjan *et al.* (2002), cited in Wong (2008); observed that concentrations of EDCs are found present at hot spots associated with manufacturing and processing industries as well as in effluent and receiving waters. Supporting this was Roslev *et al.* (1998) who highlighted that EDCs are released into aquatic environments coming from sites such as wastewater treatment plants and solid waste disposal. Hence EDCs are found in effluents and sludge. Since sewage sludge is used in forestry and agriculture, in many countries as a soil fertilizer; it can be justified to assume that EDCs are found soil systems. When food products cultivated in such soil are consumed by humans and animals, there would be a certain level of exposure.

2.5.3.2 Routes of exposure

There are various ways in which humans can be exposed to EDCs such as phthalates and synthetic hormones found in personal care products. This can happen through direct contact and use of materials containing harmful compounds, through the leaching of these compounds into other products or through environmental contamination. Other ways of exposure can be inhalation, ingestion and dermal or skin contact (Heudorf *et al.,* 2007).

In most studies conducted, it has been concluded that diet is the major route of exposure for environmental chemicals, especially phthalates. This was also shown in studies by Silva *et al*., 2003; Doull *et al*., 1999 and Huber *et al*., 1996. Li and Gu

(2006) pointed out that printing ink used on wrappers may be a route that phthalates enter into food or even through the use of PVC in food production process.

Figure 2.3 and 2.4 respectively, shows the different routes of exposure and the mechanisms of exposure of humans to phthalates which are classified as known endocrine disruptors.

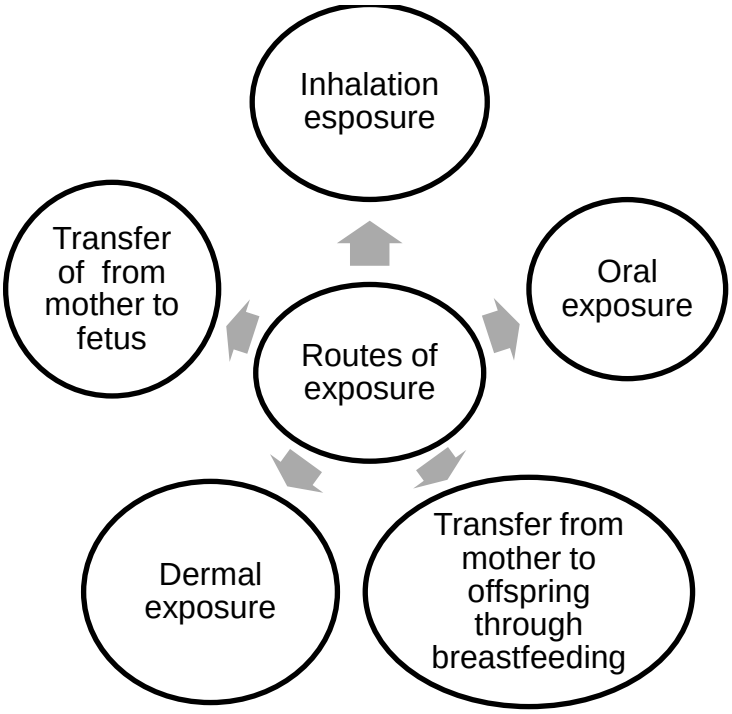

Figure 2.3 Different routes of exposure of humans to phthalates

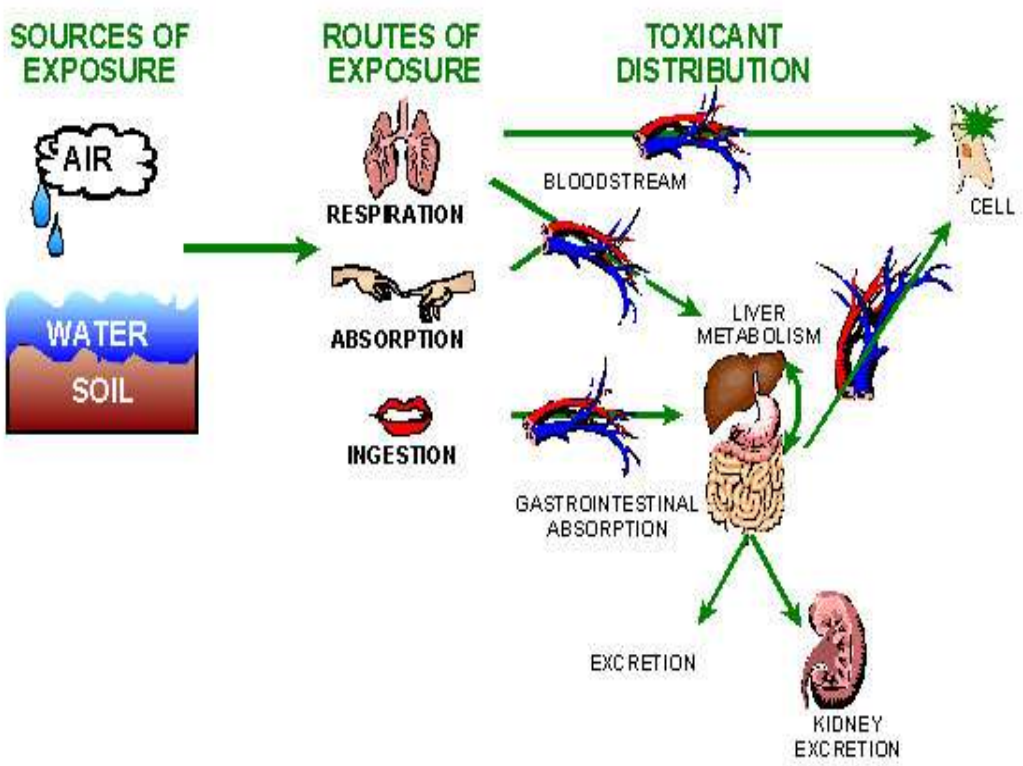

Figure 2.4 Mechanisms of exposure to environmental chemicals

(adapted from www.emcom.ca/science/pics/routes: Accessed 12 April 2010)

DEHP is mostly ingested by humans leading to exposure (Fromme *et al*., 2007 and Heudorf *et al*., 2007). Tsumura *et al*. (2001) shows the presence of phthalates in materials used in food packaging and processing, as well as gloves used in food handling. There has also been a detection of phthalates in hospital food and in hospital patients, which was concluded to be associated with food processing using PVC gloves and plastic packaging

Humans are also exposed to phthalates by using products that contain these chemicals. In 2001, Tickner *et al*. (2001) concluded that DEHP leaches in varying concentration into solution stored in PVC medical devices and hence there has to be a substitution of other materials for PVC in medical devices. This was also supported by Schettler (2006) who indicated that medical

devices containing DEHP are a source of significant exposure in a susceptible subpopulation of individuals, especially those undergoing intensive care, platelet transfusion, hemodialysis and extracorporal membrane oxygenation (ECMO) in newbo

Hauser *et al*. (2004), cited in Sathyanarayana (2008), noted that DEP and DBP in medical tablet coatings might be an important source of exposure for those ingesting enterically coated pills on consistent basis. Meanwhile, Latini (2005) supported that DEP exposure mainly occurs through dermal and inhalative exposure since it is found in hygiene products such as soap, shampoo, and conditioners.

2.5.3.3 Toxicity of plasticisers to human health

2.5.3.3.1 Background

There are about 100 plasticisers of commercial importance (Goulas *et al*., 2000). According to Till *et al*. (1982) most of these plasticisers normally possess high mobility due to their relatively low molecular weight and thus have a tendency to migrate from the packaging material into the packaged food, thereby, becoming indirect-food additives. In a study by Cheung *et al*. (2007), work by other researchers was cited; showing how phthalates can have a toxic effect in humans and animals. Much evidence shows that the lower molecular weight phthalates and their metabolites are dangerous and they disrupt the endocrine system. This then interferes with the development and reproductive system of animals and humans; by mimicking, antagonizing and even disrupting endogenous hormones and their receptors (Colón *et al*., 2000; Hauser *et al*., 2004b; Main *et al*., 2006; Morteani *et al*., 2006). As a result of these findings, according to Wang *et al*. (2000), the China National Environmental Monitoring Center and the United States Environmental Protection Agency designated phthalate esters as the priority pollutants.

These characteristics of the plasticisers become a problem in the food industry because most of the plastic-food wrappers contain plasticisers. Tice (1996) pointed out that when a plasticiser migrates from the material, it causes impermanace because of volatility, exudation, extraction or any other

influences in the application environment which then leads to the plasticiser affecting other physical properties of the polymer such as adhesion, electrical properties, flammability and toxicity. The migration of undesirable components into foods has the potential to affect product quality (Tice, 1996) and product safety (Carter, 1977).

Many studies published in the literature have shown the toxicity of plasticisers, mainly DEHP (Deisinger *et al*., 1998; Gray *et al*.,2000; Bernal, *et al*., 2002). Due to this occurrence, both the US FDA and the EU have set regulations on plasticizer use in food packaging materials (Commission of the European Communities, 1994). It is documented (Till *et al*.,1982; Goulas and Kontominas, 1996) that plasticizers such as adipates and phthalates from plasticised films easily migrate into fatty foods when there is a direct surface contact between the film and food. Straples *et al*. (1997) explained that DMPE easily leaches out and migrate into the environment through manufacture, consumption and disposal of the products. This is because like many phthalates; DMPE binds non-covalently and loosely to the products.

Different types of plastic food wrappers used in food packaging are composed of polymers which include PET, High Density Polyethylene (HDPE), PVC, Low Density Polyethylene (LDPE), Polypropylene (PP), and PS. These plastic food wrappers contain different compounds such as di (2-ethylhexyl) adipate (DEHA) and aetyltri-butyl citrate (ATBC) which might be harmful to the consumers. These compounds have been shown to disrupt normal endocrine signaling in *in vitro* and *in vivo* animal studies (Colborn *et al*., 1993) including the potential for adverse effects on reproduction and development.

The Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) (2000), conducted a study on the different phthalates. In this study, phthalates tested negative for mutagenicity. Carcinogenicity was tested for, and the activity of DEP was found to be questionable while for DINP no evidence of carcinogenicity was found, DBP seemed to be associated with tumor promoting activity, and exposure to DEHP produced hepatocellular carcinoma

in rodents. However, Harrison *et al.,* (1997) reported the mutagenicity and carcinogenicity activity of phthalates.

Several studies conducted showed that the effect of plasticisers on human health range from reproductive and endocrine systems to respiratory and dermatological problems (Lake *et al*., 1997; EC-CSTEE, 1999; Seo *et al*., 2004; Dalgaard *et al*., 2003; Wormuth *et al*., 2006; Mitaya *et al*., 2006; all cited in Bonini *et al*., 2008). In 1999, results obtained, in the different toxicological tests conducted, lead to the decision that, the addition of phthalates in plastic toys (EC, 1999) and materials for direct-food wrapping be prohibited (EC, 2002).

In the study conducted by Goulas *et al*. (2007), it was concluded that there are no known health problems imposed on the basis of a speculation that an average daily consumption of 100g halva containing 81.8mg DEHA/kg would result in a daily intake of 8.2mg of DEHA. This in turn corresponds to 0.14mg DEHA/kg body weight for a 60kg adult, a value below that of 0.3 mg DEHA/kg body weight which has been set by the EU (EC, 1993) as the tolerable daily intake (TDI) of DEHA.

2.5.4 Effect of EDCs in humans and animals

2.5.4.1 In humans in general

In the early 1990s, there were reports regarding the potential adverse human health impacts of exposure to EDCs. It was hypothesized by Safe (2004) that endocrine-active chemicals (EACs) that have caused reproduction problems in wild life, may be responsible for the increased incidence of breast cancer and disorders of the male reproductive tract. Safe (2004) conducted a research to investigate whether there is indeed a human health problem due to exposure to EDCs with reference to studies by other researchers. It was concluded that there was no correlation between organochlorine environmental contaminants and development of breast cancer. There was also no published data that supported the hypothesis. However, Doull *et al*. (1999) argues that through the results obtained by different researchers on the effect of phthalates in animals, emerging evidence suggestive of harmful

effects of phthalate exposure on the reproductive system and human health cannot be totally ignored.

2.5.4.2 Effect of EDCs in the development of foetuses

Latini *et al*. (2005) discussed a comparison of the development of male and female foetus; stating that testis formation and hormone production by the foetal testis play a vital role in the development of mammalian foetus into a male (Sharpe, 2001). However, since phthalates are classified as endocrine disruptors, they will influence the development described above. Supporting this was a report by the Centre for Devices and Radiological Health (2001), in which hormonal disruption of the foetal testis development was reported. According to Latini *et al*. (2005), in this development, the involvement of the production and activity of hormones, such as androgens and anti-mullerian hormone is required for the development of foetal testis. On the other hand, Sharpe (2001), mentions that abnormal development of the testis in foetal or neonatal life can have life-time consequences on all aspects of reproductive function in adulthood including sperm counts.

For female development, it is different from the male development in that the female development is hormone-independent. However, this development is still hugely susceptible to hormonal disruption; for example, if the female foetus is exposed to androgens, masculinisation occurs (Sharpe, 2001).

Some of the phthalate esters are toxic to the developing male reproductive system (Swan, *et al.,* 2005). Swan *et al*. (2005) aimed in the study conducted, that anogenital distance (AGD) in male infants is correlated with maternal phthalate exposure during pregnancy. In the result, all male infants appeared to be normal. Nonetheless, Gray *et al*. (2001) showed that AGD in male rats decrease and little information is known on the effect on humans since the test was only conducted in animals.

On the contrary, Benson (2009) assessed the impact of phthalate esters (DBP, DIBP, BBP, DEHP, DPP and DINP) on the development of male reproduction organs. Through the study, it was concluded that it is not likely

that humans are suffering developmental effects from current environmental exposure to these phthalate esters. Yet Gray *et al.* (2000) showed that phthalates DEHP, BBP and DINP alters sexual differentiation in male rats. The same tests were done for DEP, DMP and DOTP; with a conclusion that exposure to them does not yield any sexual differentiation.

2.5.4.3 Effects of phthalates on the reproduction of mammals

Diethyl phthalate, DBP and BBP are used in production of perfume, nail varnish, hairsprays and other personal/cosmetic uses. Blount *et al*. (2000) analysed seven urinary phthalate metabolites from a reference population in the USA, and it was found that the highest metabolites reflected exposure to DEP, DBP and BBP. This then raised a question in that inhalation and dermal exposure to these phthalates may be important routes of exposure.

In rats, DEHP was found to be a reproductive toxicant in both male and female showing that different phthalates may have different or similar effects in male and female species comparatively (Foster *et al*., 2001). For example, DBP produces developmental effects in males and reproductive tract effects in females. This was shown in a study by Foster *et al*. (2001); wherein reduced testosterone levels and increased Leydig cell numbers in rats after DBP administration were reported. In addition, Mylchreest *et al*. (1998) and Fisher *et a*l. (2003) showed that administering DBP to pregnant females induces a syndrome resembling testicular dysgenesis syndrome in the rat male offspring.

2.5.4.4 Exposure and effect of phthalates in children

Children are exposed to phthalates through medical devices, such as which pose a considerable risk of their health. Through a risk assessment conducted by the FDA on phthalates such as DEHP, in the medical setting, it was found that neonates may be exposed to phthalate, at concentrations approximately five times greater than the allowed daily tolerable intake (FDA, 2001). This was of concern to neonates who receive regular blood transfusions since DEHP which is in the PVC disposable medical devices such as gloves, IV tubing and respiratory tubing. The phthalate can leach into the blood and

subsequently into the patient. The red blood cells and plasma are typically stored in DEHP plasticised bags and administered to patients through DEHP plasticised intravenous tubes (Rais-Bahrami *et al.,* 2004). This led to the conclusion that children that go through medical procedures may represent a population at increased risk for the effects of DEHP.

For infants and children, food and food products are classified as the largest level of exposure (Sathyanarayana, *et al.,* 2008). This is because of the ability of the phthalate to leach from the PVC into food and most of the children's foods are in plastic containers. For example, in Petersen and Breindahl (2000), phthalates were found in baby food but were estimated to make up only 1% of total acceptable intake. However, several authors have documented phthalate exposure for young infants and children through breast milk, infant formula and cow's milk. In 2006, Sorensen found DEHP in both infant milk and cow's milk (Sorensen, 2006).

Sathyanarayana (2008) cited a study that was conducted by Zhu *et al*. (2006) in Canada. In this study, 86 samples of breast milk from 21 breastfeeding mothers were tested over a six month period. The results showed the presence of DEHP, DBP, and DEP in measurable concentrations, although the concentrations were not consistent within individuals and varied over time. This study just showed another risk of exposure of children to phthalates.

According to a study by Mortensen *et al*. (2005) certain phthalates contaminated breast milk. The study conducted in Denmark in 2005; where 36 samples of breast milk from healthy mothers were tested for the presence of phthalates showed that phthalates monoesters were detected. This included mono-n-butyl phthalate (MBP), monoethyl phthalate (MEP), mono-2-ethylhexyl phthalate (MEHP), monobenzyl phthalate (MBzP), and monoisononyl phthalate (MiNP) in varying concentrations. In infant milk and cow's milk, MBP and MEHP were found above detectable limit. The presence of these phthalates shows the correlation between level of exposure and the amount of phthalate excreted. Reasons for the presence of these phthalates was that breast milk is pumped using plastic breast pumps, which do contain

phthalates. Hence, as the milk is pumped, MEP and MBP leach from breast pumps into milk (Latini *et al*., 2004).

Children are also exposed through baby care products such as powder, lotion and shampoo. As shown by Stringer *et al.* (2000) that a number of phthalates are present in children's plastic toys. Stringer *et al*. (2000) studied the presence of phthalates in 72 soft PVC toys from 17 different countries and detected DINP and DEHP as the predominant phthalates. This was also proven in a study by Sathyaranayana *et al*. (2008) who stated that children are vulnerable to phthalate exposures because of their hand-to-mouth behaviours, floor play and developing nervous and reproductive systems. It as also shown that the US Consumer Product and Safety Commission found DINP in several children's toys. These studies show that the majority of soft PVC toys contain significant proportions of phthalate esters that are capable of leaching during us posing an exposure risk to infants. Another exposure of phthalates is in plastic toys which contain phthalates (Sathyanarayana, 2008). The phthalate leaches from the toys leading to children being exposed.

2.5.4.5 In animals

Boerjan *et al.* (2002) gave evidence showing that there is a relationship between contaminant exposure and compound accumulation in animal tissue, in species such as alligators, fish and monkeys.

Latini (2005) stipulated from the studies conducted by ATSDR (1995; 1997; 2001; 2002) that phthalates are animal carcinogens and can cause foetal death, malformations, testicular injury, liver injury, anti-androgenic activity, teratogenicity, peroxisome proliferation and especially reproductive toxicity in laboratory animals. These facts are only through animal testing, and there is still a limitation of information on sources and pathways of human exposure to phthalates.

2.6 Regulation and legal limits for migration of phthalates from food packaging materials

The manufacturers of packaging materials have problems with the migration limits in terms of concentrations of migrants in food (Grob, *et al*., 2006). This is because most of the time it is unknown how much food the material will be in contact with. Small amounts of food are usually in contact with more packaging material; resulting in higher surface area per volume and, therefore, the smaller the quantity the higher the concentrations in food. Since the contact of these compounds to food might result in health implications to the consumer, the industry wants to be protected against extreme requirements in the case of a small amount of food in contact with an extremely large packaging surface area. They argue that foods packed in small portions are usually consumed in small amounts; hence the consumer experiences no harm. It is, therefore, vital to indicate specific migration limit (SML) and the overall migration limit (OML) as limit per food contact surface area (Grob, *et al*., 2006).

European legislation regulates migration from food contact materials, such as packaging, into foods by an OML applicable to the total of the migrating material and SMLs referring to individual substances or groups of substances. In Grob *et* al. (2007), it was highlighted that the European legislation also specifies that OM and SM should be determined as concentrations in food if the packaging material can be filled (known surface area/volume ratio) and has a volume between 0.5 and 10 l (Directive 2002/72/EC, Article 2). The EU Directives on plastics in contact with food, presently Directive 2002/72/EC and its amendments, are the leading legislation in Europe, complimented by Directives regarding technical aspects. Currently a new regulation, the so called ‘‘Super Regulation’’, is in preparation, combining the relevant Directives and further developing them (Grob *et al*., 2007).

The EU has specified a tolerable daily intake (TDI) for certain plasticisers. For example, “the TDI’s for DEHP and DINP + DIDP are 0.05 and 0.15 mg/kg body weight respectively, which the SLM of 3 and 9 mg/kg would be derived

provided that the whole TDI were allotted to food contact material. For DEHA, Directive 2002/72 specifies an SLM of 18 mg/kg (SCF, 1999, cited in Frakhauser-Noti and Grob, 2006)".

In the UK, the acceptable concentrations (TDI) for BBP were set at 0.1 mg/kg body weight/day; DBP at 0.05 mg/kg body weight/day and DEP at 0.2 mg/kg body weight/day (MAFF, 1996). In Europe, the total tolerable daily intake (TTDI) per person of total phthalate esters has been estimated to be 0.3 mg/kg body weight (Petersen, and Nielsen, 1995).
The National Legislation is relevant to the food packaging and should be taken into account. The EU Directive 97/48/EC, which is the second amendment of Directive 82/711/EEC, specifies the test conditions of samples; that "if the product is pasteurised or sterilised, the conditions should be reproduced; migration during long-term storage is simulated by 10 days at 40˚C". It can, therefore, be seen that there are rules that are stipulated by the National legislation that should be followed.

2.7 Presence of microorganisms and pathogens in food

Microorganisms play different roles in food. They can be desirable and used for food bio-processing, or they can be undesirable which means they can cause food borne diseases and food spoilage. Spoilage in different types of food can be identified through changes in colour, odor and texture; formation of slime; accumulation of gas or foam; and release of liquids (Ray, 2001). Ray (2001) further highlights that the presence of undesirable microorganisms in the food can be responsible for the spoilage. The spoilage of these foods is not only because of the presence of these microorganisms but also through contamination that occurs during the different stages of processing and preserving the food.

The current study focuses on processed foods. Ray (2001) highlights that many processed foods generally contain several types of moulds, yeasts and bacteria that have the ability to reproduce and cause spoilage. Some of the main food spoilage bacteria include psychrotrophs, thermophillic bacteria and

acidophillic bacteria. Foods can be grouped as perishable (spoil in days), semi-perishable (have a relatively long shelf life, few weeks or months), and non-perishable (have a extraordinarily long shelf life, several months or years). Hence, these factors are taken into consideration when determining how long a food sample will take to spoil.

According to Centres for Disease Control (CDC, 2007) and Eurosurveillance (2007); cited in Jofre *et al*. (2008); *Listeria monocytogenes (L. monocytogenes), Salmonella* and *Staphylococcus aureus (S. aureus)* are food-borne pathogens and outbreaks associated with these pathogens have been reported in meat products.

L. monocytogenes are problematic for the food industry. These bacteria have been found to be able to grow in many chilled vacuum-packed, cooked perishable, cured meats (Bersot *et al*., 2008). In Chile, *L. monocytogenes* were found in ice cream, soft cheese, processed meat products and crustaceous shellfish (Cordano and Rocourt, 2001). In a number of literature, *L. monocytogenes* have been found to grow in a number of foods. This was attributed to this organism being widespread in the environment (Farber & Peterkin, 1991) and its ability to grow in a wide range of temperature; i.e. between 3ºC (and lower) and 45ºC (Harwig *et al*., 1991). This organism causes a disease known as listeriosis (Rocourt and Bille, 1997), with a fatality of 20-30%.

S. aureus, like *L. monocytogenes*, has the ability to grow at a wide range of temperatures and pH. Bean *et al*. (1990) considered staphylococcal food poisoning, which is caused by the toxins of *S. aureus*, to be one of the most frequently occurring food borne disease worldwide. The staphylococcal toxins cause gastroenteritis in humans.

Salmonella causes a disease known as salmonellosis in humans. Food that originate from animals; such as beef, chicken, turkey, pork, eggs, milk and products made from them; have been associated with a large number of outbreaks (Ray, 2001). According to Lehmacher *et al*. (1995), an outbreak of

human salmonellosis occurred in Germany. This affected over 1000 cases including children. Salmonellae are said to be natural inhabitants of the gastrointestinal tracts of domesticated and wild animals, birds and pets including turtles and frogs) and insects. According to a review conducted by Sweat *et al*. (2008), turtles can cause human *Salmonella* infection. They looked at a case study which showed that two girls who had been swimming in a pool where two turtles swam, became sick showing symptoms similar to those of salmonellosis. Laboratory tests were conducted and it was discovered that in a water sample collected from the turtle habitat, *Salmonella Paratyphi B* var was present. This then showed that turtles do indeed cause human *Salmonella* infection.

Contaminants are substances that have not been deliberately added to food and during the stages of production, packaging, transport or holding, these substances can enter into food. They also might result from environmental contamination. Since contamination generally has a negative impact on the quality of food and may imply a risk to human health, the EU has taken measures to minimize contaminants in foodstuffs. Reij and den Aantrekker (2004) conducted a study to evaluate whether the recontamination is responsible for the presence of pathogens in foods. It was demonstrated through a review of many outbreaks that the presence of vegetative pathogens such as *Salmonella* spp. or *Listeria monocytogenes* in the consumed products was frequently due to post-process recontamination. Supporting this was a survey on control of foodborne infections and intoxications in Europe by WHO (1995). It showed that almost 25% of the food-borne outbreaks could be traced back to recontamination. Other contributing factors to the presence of pathogens in prepared foods were said to be insufficient hygiene (1.6%), cross-contamination (3.6%), processing or storage in inadequate rooms (4.2%), contaminated equipment (5.7%) and contamination by personnel (9.2%).

Before processed foods are commercialised, they are heat treated so as to ensure safety and long shelf-life. According to von Holy *et al*. (1992), processed meats are heat treated to a core temperature of 70ºC or even

above. These results in a virtually sterile product, however, recontamination by a wide range of microorganisms occurs. Giovannacci *et al*. (2001), cited in Reij and den Aantrekker (2004), stated that micro-flora which resides on food contact surfaces of processing equipment have an impact on the transfer and recontamination of meat by pathogens such as *S. aureus, L. monocytogenes* or *Salmonella*. It was discovered that containers, pumps or tanks used for holding or transporting unprocessed raw materials, such as raw meat and poultry or unpasteurised liquid egg, occasionally have subsequently been used for processed products without any cleaning (Morgan *et al*., 1993; Evans *et al*., 1996; Hennessy *et al*., 1996; Llewellyn *et al*., 1998; all cited in Reij and den Aantrekker, 2004). This supports the study by WHO (1995) in which 1.6% of the presence of pathogens in food was due to insufficient hygiene. Lack of effective and adequate cleaning and disinfection of the processing equipment also results in the presence of these pathogens in food.

In a previous study by Borch *et al*. (1996), the bacterial spoilage of meat and meat products was investigated. It was explained that a certain maximum acceptable bacterial level or an acceptable off-odour, off-flavour or appearance marks spoilage of meat and its products. This is however different these days because most of the meat and its products are marked with best by or expiry dates after which they are discarded from the shelves and considered not safe for human consumption. The initial number of microorganisms present determines the shelf-life of the product.

2.8 Techniques for the extraction of phthalate esters and determination of microbes in food samples

The procedure for the analysis of chemicals in food follows the following steps: pre-treatment, extraction, clean-up and instrumental analysis.

2.8.1 Sample pre-treatment

This involves cutting samples into smaller pieces by homogenising with pestle and mortar. This was done in the analysis of the migration of DEHA from PVC into hard and soft cheeses (Goulas *et al*., 2000). This was done so as to

increase the surface area for the extraction step and to increase the extractability of the analytes. Tsumura *et al*. (2002) used a homogeniser to increase the surface of the different food samples, which were analysed for the presents of phthalates. Goulas and Kontaminas (1996) evaluated the migration of plasticisers into chicken and conducted sample pre-treatment wrapping chicken with PVC cling film. This ensures full contact of the chicken to the PVC wrapper. A similar study was conducted by Goulas *et al*. (2000) on cheese. Sample pre-treatment also involved wrapping the cheese with PVC wrapper.

2.8.2 Extraction

Extraction is usually the second step in the analysis of environmental chemicals as mentioned by Lundstedt *et al*. (2003) and it is performed to release contaminants from the solid matrix and transfer them quantitatively to another medium which in most cases it is an organic solvent. In the current study, the organic solvent hexane was used. After extraction, the phthalates samples are collected and prior to instrumental analysis, the solvent volume is reduced. There are various methods which are used in extraction in order to detect the presence of phthalates. These methods include Soxhlet, supercritical fluid extraction pressurized liquid extraction (PLE), ultrasonic extraction, microwave-assisted extraction (MAE), a solid-fluid fluidizing series extraction procedure and solid-phase micro-extraction (SPME).

According to Garcia *et al*. (2006), extraction is done in order to separate the migrants from the polymeric unit. In the same study, it was also stated that the analysis of volatile and non-volatile samples is different. For volatile migrants, headspace GC technique is appropriate while for non-volatile samples; a liquid extraction step can be used. In Jaillais *et al*. (1999), solid-phase microextraction (SPME) was said to be a technique that allows extraction and concentration of volatiles or semi-volatiles from the sample matrix (usually a water sample also used in headspace) (Alpendurada, 2000; Kotowska and Garbowska, 2006).

With regards to Soxhlet extraction, Portugal *et al*. (1999) points out that the Soxhlet extraction method requires extremely long extraction times (6-8 hours) and involves the use of environmentally hazardous solvents, which then imposes a high cost of analysis. However in most recent years, instrumental techniques have been developed which save both time and the solvent. Representative examples of these new extraction techniques are ultrasonic extraction, supercritical fluid extraction, PLE, and MAE, and recently, a solid-fluid fluidising series extraction procedure, which provides for a relative simple and cost-effective alternative. The main advantage of Soxhlet extraction is that the sample phase is always in contact with fresh solvent and due to moderate extraction conditions, compounds are not decomposed.

In literature, in order to detect phthalate esters at sub parts per million (ppm) levels, various liquid–liquid extraction (LLE) approaches have been used for isolation from aqueous samples (Zhu *et al*., 2006; Yasuhara *et al*., 1997; all cited in Carlo, *et al*., 2008). This technique has been considered as an alternative for the determination of phthalate esters in liquid samples, because the risk of contamination during sample handling can be significantly reduced, but it appears not applicable to wine analysis because in this matrix the phthalate esters partition in the liquid phase is enhanced by the high percentage of ethanol.

Solid phase extraction has been used in sample preparation of DEHA and also in fatty foods such as animal tissue, fats and high fat content cheese. The sample is blended in an appropriate solvent such as hexane or dichloromethane and then allowed to pass through a florisil column (Page and Lacroix, 1995). According to Brossa *et al*. (2002), solid phase extraction can also be used to extract DEHA in water samples.

Zygoura *et al*. (2005) used cold point extraction coupled with microwave assisted back extraction to determine the migration levels of DEHA and ATBC from a PVC food packaging film into aqueous stimulants. This methodology was concluded to be effective for the determination of migration levels.

It is broadly accepted that the solvent used should dissolve the target compound and also swell the polymeric matrix. Polymer swelling data are widely available in the literature, but the solubility of the selected migrants in extraction solvents are incompletely documented and must be estimated on the basis of the nature of analyte and extractant. Several combinations of solvent/analyte/polymer have been used. Dichloromethane has been used to determine Irganox 1076 and DEHA in multilayer materials (PP/EVA/EVOH) and PVC; acetonitrile for Chimassorb 81 and Irganox 1076 in PET; tetrahydrofuran for Chimassorb 81 and Uvitex OB in HDPE (high density polyethylene) and PP, iso-octane for DEHA in PVC and diethyl ether for Irganox 1076 and DEHA.

Extraction procedures are usually carried out by hand shaking, although sometimes ultrasonic maceration or Soxhlet is used to improve the process (Nerı´n *et al*., 1996; O'Brien *et al*., 1997; Peterson *et al*., 2004; Ulsaker and Teien, 1992). Regarding other food contact materials, benzophenone was extracted from paper and board with absolute ethanol (Summerfield & Cooper, 2001; Triantafyllou *et al*., 2002). Extractions with supercritical fluid or microwave-assisted were used for Irganox 1076 and Chimassorb 81 in PE (Salafranca *et a*l, 1999), and DEHA in PVC (Cano *et al*., 2002; Guerra, Marı´n *et al*., 2002).

2.8.3 Clean-up

This step is performed to co-extract compounds that could interfere during subsequent analysis and separate the different analytes before analysis. The different methods that are used for the clean-up process through adsorption chromatography include open-column chromatography, high performance liquid chromatography and solid-phase extraction (SPE).

2.8.4 Instrumental Analysis

This is an important step in the methodology. It is done in order to separate, identify and quantify the individual analytes in the sample. There are various

methods which are used in polymer analysis. One of those methods is the pyrolysis gas chromatography (Py-GC). This method was used by Wang (2000) in the analysis of polymer additives. This technique uses thermal energy (pyrolysis) to breakdown a polymeric chain to monomers, oligomers and other fragments. The pyrolysates are then separated by GC and detected by appropriate detectors. The pyrolysis gas chromatography method can be used to quantitatively and qualitatively analyse plasticisers. There are advantages of this technique. Plasticisers can be determined in polymers simultaneously with polymer composition and microstructure analysis. There is no sample preparation required and all the information can be obtained within one experiment (Eiceman *et al*., 1998).

GC involves two types of detectors: flame ionization detector (FID) and mass spectrometry (MS) or mass-selective detection. FID is one that is frequently used for quantitative analysis of pyrolysates while MS is mainly used for identification. According to van Zuydam (2007), GC has become more popular due to its high selectivity, good precision and resolution.

In Silva *et al*. (2006), many methods which can be used in polymer analysis where explored. This Py-GC, high performance liquid chromatography (HPLC), GC (FID and MS) and SPE (supercritical fluid chromatography). However, in all these methods, the method of choice for the current study is GC-FID. This method has been reported in various literatures (Goulas *et al*., 2007; Goulas and Kontominas, 1996; Goulas *et al*., 2000) for the determination of phthalates esters.

2.8.5 Microbiological

There are many methods used to determine the presence of pathogens in food stuffs. Steps used in analysis include sample preparation, plating techniques, counting and identification test kits. The limitation with these methods is that they are laborious, tedious and time consuming in the counting of the observed growth on the plated agar. However, there are counting methods such as flow cytometry and the direct epifluorescent filter

technique which are suitable techniques for rapid detection of microorganisms, especially in fluids (de Boer and Beumer, 1999).

Some conventional methods used in detection of microorganisms in food include blending of the food product with a selective enrichment medium to increase the population of the target organism; plating onto selective or differential agar plates to isolate pure cultures; and examining the cultures by phenotypic analysis or metabolic fingerprinting (monitoring of carbon or nitrogen utilization). These methods are good but they also have drawbacks. When detecting bacteria using these methods, a lot of time is consumed, they are also labour-intensive and take 2–3 days for results to be obtained, and up to 7–10 days for confirmation (WHO, 1995).

According to Naravaneni and Jamil (2005), molecular techniques can also be used for rapid identification of foodborne pathogens. Methods such as cloning and recombinant DNA techniques have been said to have brought a revolution in the detection of pathogens in food. Naravaneni and Jamil (2005) reffered to work by Rasmussen *et al.* (1994) and Cohen *et al.* (1993) as having brought great developments in rapidly identifying bacterial strains through gene probing techniques. These techniques were said to be of advantage because they do not require the isolation of pure cultures.

Important developments in the use of nucleic acid-based assays for the detection and subtyping of foodborne pathogens have been shown in literature. The sensitivity of these methods has been significantly increased by the use of the polymerase chain reaction (PCR) and other amplification techniques. PCR uses *in vitro* amplification of specific segments of DNA by using a pair of primers (Nguyen *et al*., 1994; cited in Naravaneni and Jamil, 2005). According to Finlay and Falkow (1988) and Bej *et al*. (1994), PCR can be used to amplify genes specific to taxonomic groups of bacteria and also to detect genes involved in the virulence of food-borne bacteria. Since food pathogens are found in very low numbers in the contaminated food, it becomes one of the limitations in detecting the microbes. However, with PCR

even one molecule of target DNA can be detected. It was then concluded that by amplifying a sequence that is unique to the pathogenic microorganism of interest, the *in vitro* amplification methods can be used to indirectly detect extremely low concentrations of microbes.

CHAPTER 3

METHODOLOGY

The methodology and experimental procedures used in the study are presented in this chapter. The sampling procedure is described. Food samples were randomly purchased for phthalate esters analysis and microbiological analysis. The phthalate analysis involved a three-step procedure: sample preparation, extraction by Soxhlet extraction and analysis by gas chromatography (GC-FID). This was followed by a microbiological analysis of food samples from all the four regions of Tswane metropolitan area, i.e. east, west, north and south. The steps are described in the next sections.

3.1 Overview of sampling area

The sampling site for this study was the Tshwane Metropolitan Area in Pretoria, located in the northern part of Gauteng Province, South Africa. It is one of the country's three capital cities, it being the administrative capital, Cape Town, the legislative capital and Bloemfontein being the judicial capital. This city is contained within the City of Tshwane Metropolitan Municipality with a population of 600,000 to 1.2 million (Wikipedia, n.d.; accessed 14 January 2011). Pretoria was selected as the sampling area because of the ease in accessibility.

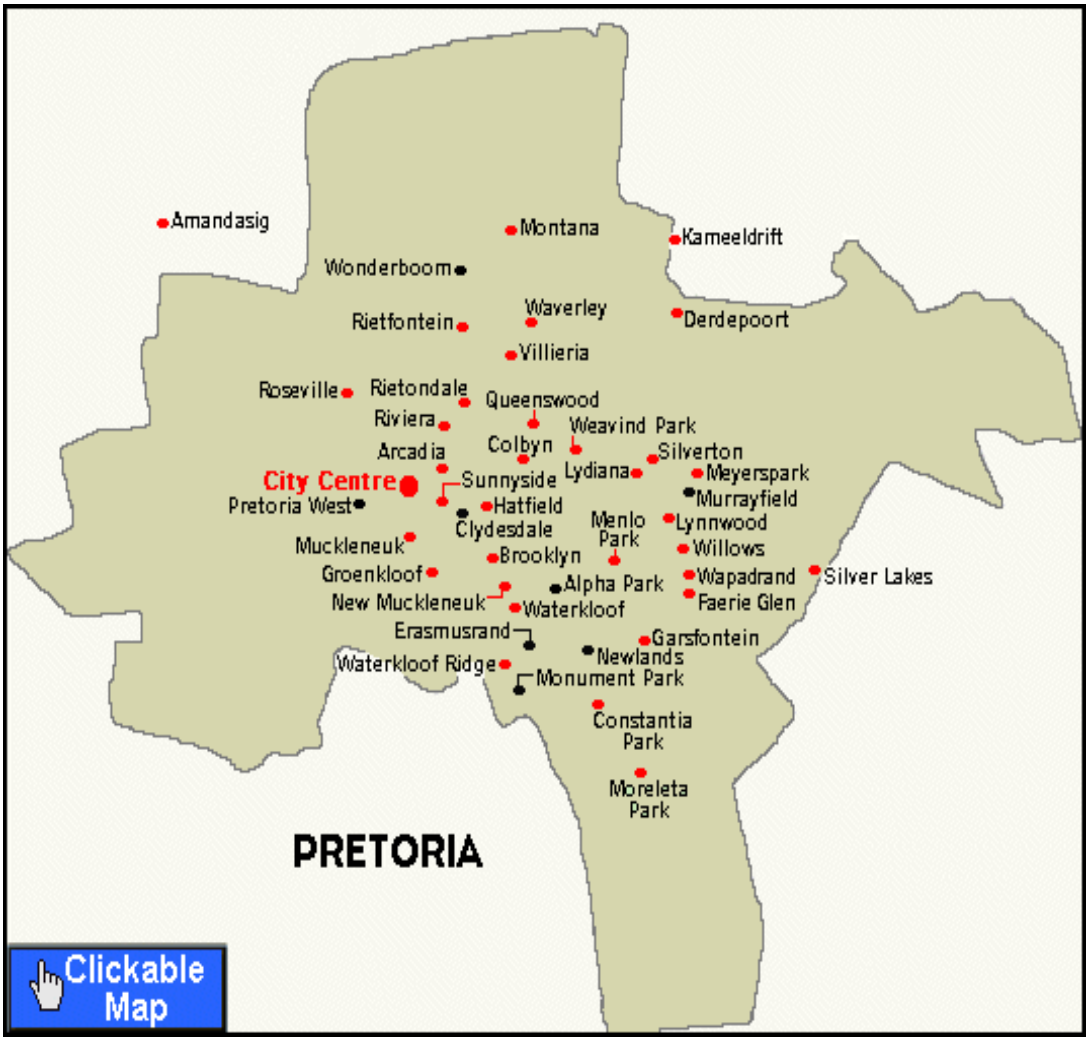

Figure 3.1 Pictorial presentation of Pretoria

Source: Wikipedia, n.d (Accessed 14 January 2011).

3.2 Samples

The three selected food types that were used this study include: cheese, polony and vienna. These samples were purchased from selected departmental stores. However, each sample had to be wrapped in plastic with direct contact between the food and the plastic wrapper. A total of 30 cheese samples, 31 polony samples and 20 vienna samples were analysed. The departmental stores in which samples were purchased were also randomly picked.

3.3 Study area

This study was conducted in four study areas, Pretoria North, South, West and East. Different samples were purchased from different departmental stores in the four regions of Tshwane Metropolis. Figure 3.2 below indicate the general orientation of the study sites in Thswane metropolitan Area of gauteng Province.

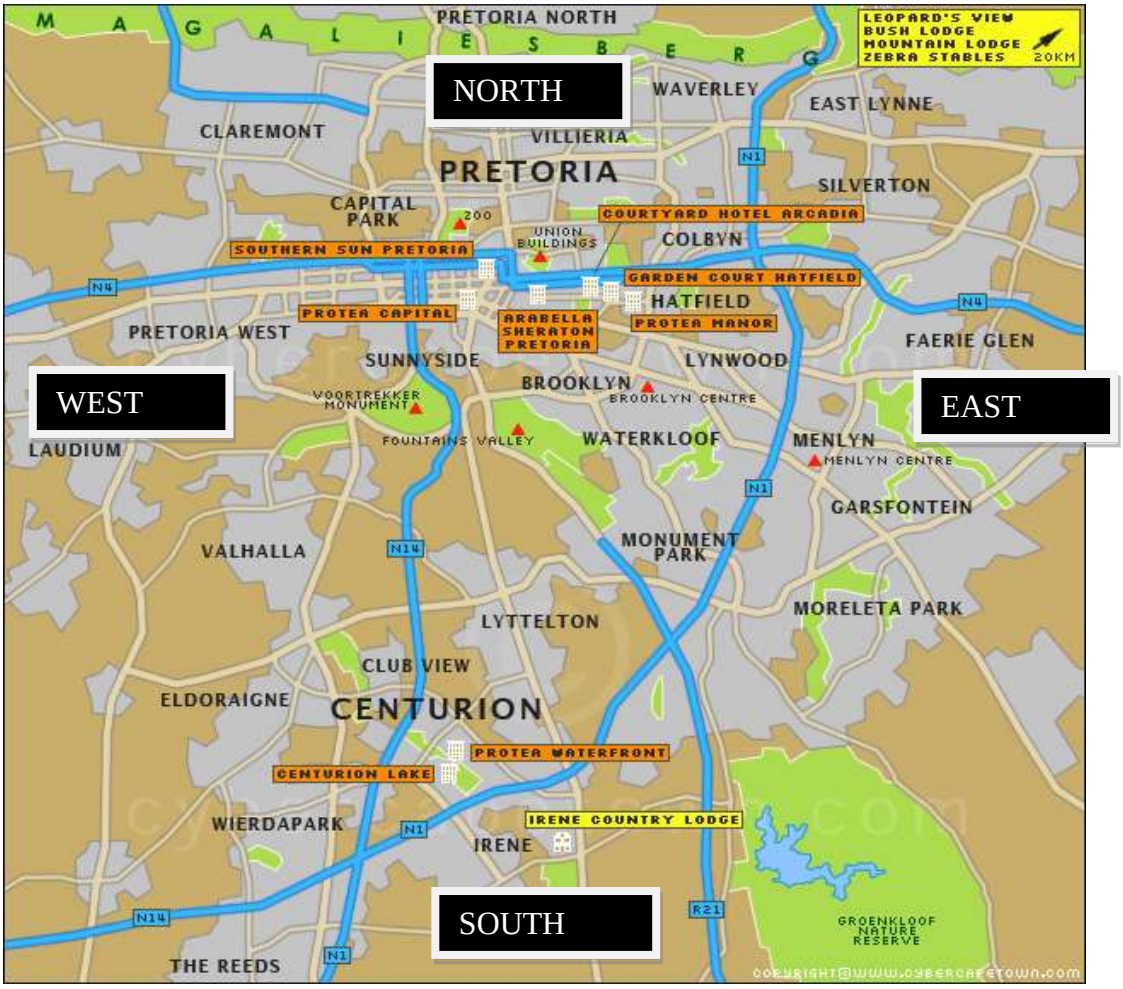

Figure 3.2 Map showing the four study areas: Pretoria North, South, West and East

Source: Wikipedia, n.d (Accessed 14 January 2011).

In all study areas, different departmental store was chosen and samples were purchased at different time span in the year 2009 to 2010. The purchased samples; cheese, polony and vienna, had to be wrapped with plastic. They were then stored in the refrigerator until analysis.

3.4 Sampling protocol

The samples were purchased from selected departmental stores in the four regions of Tshwane metropolitan area during 2009-2010. The selected samples can be classified under foodstuffs which are consumed almost on a daily basis in various households; hence they were selected for this study. All samples that were purchased were wrapped in PVC cling film, which ensured a direct contact of the plastic and the food sample. Samples were stored in the refrigerator at 4ºC and analysis was conducted within 48hrs from date of purchase.

3.5 Sample pre-treatment

Polony and vienna samples were cut into smaller particles and transferred into a Petri dish. These samples were then dried in the oven at 50ºC for 24h. The samples were allowed to cool and then using a mortar and pestle, they were homogenised, thus increasing the surface area. However, with cheese samples, the outer surfaces were removed to a depth of about 5mm and then weighed and analysed. About 1g was weighed of each sample and further analysed as described below.

3.6 Extraction

The most common way to incorporate plasticisers into polymers is by blending, that is, by physically mixing with the polymer molecules. When analysing the plasticisers, separation from polymer by solvent extraction is conducted. After separating the plasticiser from the polymer, the plasticiser-containing portion can be further analysed by proper chromatographic methods with the appropriate detection (Silva *et al.,* 2006).

3.6.1 Soxhlet extraction

All samples of the packaging materials were prepared in duplicates. Following the sample pre-treatment described in section 3.5, about 1g of each powdered sample was weighed and transferred into an extraction thimble and transferred to a Soxhlet apparatus. Extraction solvent (hexane, 120 ml) was added to the apparatus and the extraction of the sample was done for 4

hours. Extraction was done at moderate heat (60-80ºC). The condensers of the Soxhlet apparatus were connected to a tap water supply to ensure that there is no loss of volatiles. After the extractions were done, the extracts were allowed to cool to room temperature.

3.6.2 Quality assurance/recovery study

To ensure applicability of the method used, quality assurance was carried out through recovery procedure of added known standards of the plasticisers to pre-analysed samples. Extraction of plasticiser, separation of the alcoholic constituent of plasticiser, and GC analysis were conducted using the same procedure as for experimental samples.

A solution of the mixture of standards was prepared with a concentration of 1mg/l. Food samples (polony, vienna, cheese) were spiked in triplicate with 1ml of 1mg/l mixtures of phthalates. Each spiked food sample was extracted as described in the Soxhlet extraction step below. The extracts were then run on the GC using the optimum conditions described above. The recovery factors were then determined.

3.6.3 Column Chromatographic clean-up procedure

Using a ratoevaporator, extracts were evaporated to about 5ml. Each extracted solution was allowed to pass through a florisil column and eluted with 20ml of hexane. The solutions were then evaporated to 2ml and transferred to a sample vial. The samples were refrigerated at 4ºC until they were analysed by gas chromatography-flame ionization detector (GC-FID). The internal standard was added into each extracted solution before GC analysis. Statistical analyses were performed using MedCalc for Windows, version 12.0.0.0 (MedCalc Software, Mariakerke, Belgium).

3.7 Phthalate analysis

Phthalate analysis was conducted using the Perkin Elmer Autosystem Clarus 600 Gas Chromatograph, with FID detector and capillary column (30m length; 0.25mm internal diameter with 0.25µm film thickness) supplied by Perkin

Elmer SA (Pty.) Ltd., Cresta, Johannesburg, South Africa. The gas chromatograph had a built-in autosampler, with dual column system for both FID and ECD.

The oven inlet temperature, carrier gas flow and detector temperature were programmed as follows: the oven was heated from 70ºC to 150ºC at 35º/min, hold for 1min then heated to 290ºC at 30ºC/min with hold time of 1min. The total run time was 9.95 min. Injection volume was 1µl and helium was used as the carrier gas at 50 kPa with set point of 1ml/min. Injection was in split mode at 20ml/min. Injector temperature was 250ºC and the detector temperature was 330ºC.

The reference materials which were used are: Di-2-ethyl hexyl adipate (DEHA), Di-methyl phthalate (DMP), Benzyl-butyl phthalate (BBP), Di-n-butyl phthalate (DnBP), and Dibutyl phthalate (DBP). The internal standard used was 1-chlorotetradecane. All standards and the internal standard were obtained from Merck Pty. Ltd., Johannesburg, South Africa. All standards were more than 98% pure when analysed by capillary GC. Standards were run to establish the elution times of the phthalate plasticisers. The elution times were then used to determine the presence of the phthalates in each tested sample.

All glassware was rinsed with acetone immediately prior to use and contact of food and the food extracts with material other than glass, stainless steel or their original packaging was avoided and all costs. The stock solution 1000mg/l for each ester was prepared in a 100ml volumetric flask with hexane and diluted as appropriate and stored at 4ºC. All stock solutions were prepared using glass containers. A standard mixture of the anaylates (100mg/l) in hexane was used for preparations of the calibrating solutions. The concentration of the calibration solution ranged from 0.2-0.8 mg/l.

The retention times (min) were then optimised using the optimised GC conditions. The response factors were calculated using a mixture of the phthalate esters and internal standard (1-chlorotetradecane) 100mg/l concentration with 10 replicate injections (1µl). The formula used was as

follows: area of the peak of phthalate ester/area of the peak of internal standard.

3.8 Analysis of the presence of food pathogens and spoilers in selected food samples

3.8.1 Chemicals and reagents for microbial analysis

The following reagents were used in the microbiological analysis of the food samples: Hexane, Crystal violet neutral red bile lactose (VRBL) agar, Brilliant green lactose bile (BGLB) broth, Baird-Parker (BP) agar, UVM1 broth, Fraser broth, MOX agar medium, PALCAM agar medium, one tube of *L. monocytogenes* overnight culture (positive control), one tube of *Enterococcus faecalis* overnight culture (negative control), TSAYE agar medium, lactose broth, selenite cystine broth, tetrathionate broth, Bismuth sulfite (BS) agar medium, Hektoen enteric (HE) agar medium, xylose lysine desoxycholate (XLD) agar medium, tryptone water, Kovac's reagent, Durham tubes,

3.8.2 Glassware and equipment

The following glassware and equipment were used in the microbiological analysis of the food samples: Incubator, Petri dishes, pipettes (1ml), colony-counting equipment, test tubes, Durham tubes, inoculating loop, volumetric flasks (100ml), stomacher bag, pipettes (1ml), micro pipette, yeast and mould Petrifilms.

3.8.3 Sample collection for microbial analysis

The total number of samples analysed for the presence of pathogens and food spoilers was 81 out of a total of 114 samples purchased from the supermarket. Few samples were analysed because of the financial constrains. The microbiological methods used adopted the ISO 17025 standard and SANAS (South African National Accreditation System) accredited microbiological test methods.

Table 3.1 below shows the microbiological test conducted for the analysis of the presence of specific microorganisms in the food samples from four Tshwane Metropolitan regions.

Table 3.1 Microbiological test conducted for the analysis

Polony	Vienna	Cheese
Total microbial activity (TMA)	TMA	TMA
Coliforms	Coliforms	Coliforms
Staphlococcus aureus	*Staphlococcus aureus*	*Staphlococcus aureus*
Listeria monocytogenes	*Listeria monocytogenes*	*Listeria monocytogenes*
Salmonella	*Salmonella*	*Salmonella*
Escherichia coli	*Escherichia coli*	*Escherichia coli*
Yeast and mould	Yeast and mould	Yeast and mould

3.9 Microbiological tests/analysis

3.9.1 Total microbial activity (TMA)

Food sample at 11g was weighed into a sterile petri dish and transferred to a blender. Ninety-nine millilitres of peptone water were added in the case of polony and vienna, while peptone water with 2% trisodium citrate at 40ºC was used for cheese. The homogenate was obtained by blending for 2min, making a 10^{-1} dilution of the original sample. Further dilutions up to 10^{-7} were prepared. About 0.1ml of the dilutions was pipetted into plate count agar (PCA) plates in duplicate using a sterile glass rod. The plates where inverted and incubated at 30°C for 48hrs. Colonies were counted and CFU/ml was determined. An automated colony counter was used. Each agar plate, which showed growth, was placed under the light of the colony counter and the colonies were counted.

3.9.2 Detection of coliforms and *E. coli*

Food sample at 11g was weighed and homogenised in blender for 2min using 99ml of peptone salt diluents making a 10^{-1} dilution. Additional dilutions were prepared by pipetting 1ml of 10^{-1} dilution into 9ml peptone salt diluents tubes making a 10^{-2} dilution: further dilutions were prepared following similar procedure: 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. About 1ml of each diluted solution was inoculated in duplicate in sterile Petri dishes. Fifteen millilitres of VRBL medium at 45-47ºC was added in each Petri dish. The plates were allowed to solidify on flat surface and overlaid with 5ml VRB medium. The plates were invert and incubated at 35ºC for 24h. Plates with 10 or more colonies were selected and colonies counted using a colony counter.

The purplish red colonies with a diameter of at least 0.5mm and sometimes surrounded by a reddish zone of precipitated bile are considered as typical colonies of coliforms and do not require further confirmation. However, a confirmation test was conducted as follows:

Five colonies of each atypical type were inoculated into tubes of brilliant green lactose bile (BGLB) broth with Durham tube and incubated at 30ºC for 24hr. Colonies that showed gas formation in the Durham tube were considered as coliforms.

3.9.3 Detection of *Escherichia coli*

Food at 11g was weighed and homogenate was obtained through blending with 99ml of peptone salt diluents for 2min making a 10^{-1} dilution. About 10ml of dilution 10^{-1} was inoculated in BG broth fitted with Durham tube and incubated for 48hr in a water bath at 44ºC. The presence of gas was observed and 0.2ml inoculum was transferred to tryptone water tube and incubated for 24hr in water bath at 44ºC. The tryptone water was tested for indole production by adding 0.5ml of Kovac's reagent. Formation of rose coloured ring at interface of two liquids indicated the presence of indole and confirmation that *E. coli* is present.

3.9.4 Detection of *Staphylococcus aureus*

Food at 11g weighed into a blender and homogenised for 2min using 99ml of peptone water forming a 10^{-1} dilution. Additional dilutions using 9ml peptone water tubes were prepared: 10^{-2}, 10^{-3}. Inoculations of 0.4, 0.3, and 0.3ml of 10^{-1} dilution into three Baird-Parker (BP) agar were conducted. The same step for remaining dilution was repeated. The inocula were spread using a sterile glass rod then plates inverted and incubate at 35ºC for 48hr.

Observations of four types of presumptive Staphylococcal colonies was conducted:

Type 1. Convex, entire, shiny black surrounded by clear zones extending into the opaque medium.

Type 2. Convex, entire, shiny black without well defined clear zones.

Type 3. Dark grey colonies similar to type 1.

Type 4. Dark grey colonies similar to type 2.

3.9.5 Detection of *Listeria monocytogenes*

Food sample at 25g was weighed into a stomacher bag and 225ml of UVM1 added, followed by incubation at 30ºC for 24hr. After incubation, 1ml was transferred into 9ml of Fraser broth and incubated at 35ºC for 24hr. The broth mixture was then streaked (three phase) onto selective media plates: three plates of the MOX and three plates of PALCAM agar media. A two-phase streak was performed for the positive control culture (*L. monocytogenes*) and the negative control culture (*Enterococcus faecalis*). All plates were incubated at 35ºC for 24hr. The colonies were examined on the selective-differential agar plates by picking a portion of the marked presumptive *Listeria* colony in MOX agar plate and streaking on one-half of the TSAYE agar plate. The step was repeated for colonies on PALCAM agar plate. The TSAYE plates were

incubated at 35ºC for 24hr and checked for growth of presumptive *Listeria* colonies.

3.9.6 Detection of *Salmonella*

A 25g food sample was weighed into a stomacher bag and 225ml of lactose broth was added and stomached for 2min. The bag was incubated at 35ºC for 24hr and 1ml of enriched food sample was transferred into the 10ml selenite cystine broth tube (SC enrichment). Another 1ml was transferred into the 10ml tetrathionate broth (TT enrichment) and the tubes were incubated at 35ºC for 24hr. A two-phase streak of SC enrichment onto one half of BS, HE, and XLD plates was done and repeat also for TT enrichment onto the other half of the BS, HE, and XLD plates. The plates were incubated at 35ºC for 24hr and BS, HE and XLD agar plates were observed for typical Salmonella colonies. The expected morphology of Salmonella on Bismuth sulfite (BS) agar was brown, gray, or black colonies, which can sometimes have a metallic sheen. Surrounding medium is usually brown at first, but with increased incubation, they may turn black, producing the so-called halo effect. On Hektoen enteric (HE) agar the colonies expected were blue-green to blue colonies in color with or without black centres. Many cultures of *Salmonella* may produce colonies with large, glossy black centres or may appear as almost completely black colonies. Lastly, on xylose lysine desoxycholate (XLD) agar pink colonies with or without black centres were expected.

3.9.7 Detection of Yeast and mould

Food sample of 25g was weighed into a stomacher bag and 225ml of peptone water was added and stomached for 2min. Dilutions of 10^{-2} and 10^{-3} were prepared by dispensing 1ml of the 10^{-1} solution into 9ml peptone water. A transfer of 1ml of each diluted sample to the surface of the Petrifilm was conducted. Using the glass spreader the inocula were spread uniformly over a circular area and left to stand for 1min for the gel to solidify. Plates were incubated at 22-25ºC for 5 days. Plates were then observed for growth of yeast and mould.

3.10 Limits for the amount of microorganisms in food

The Department of Health in South Africa has set limits for the amount of microorganisms or pathogens that can be found in different food types according to categories. Table 3.2 below, lists the limits that are set for cheese, polony and vienna. These limits are used as standards for the comparison with the obtained results after microbiological testing is conducted.

Table 3.2 Guidelines of environmental health officers on the interpretation of microbiological analysis data of food by the South African Department of Health

Analysis	Limits
Cheese	
Coliforms	1000 cfu/g
E. coli	0 cfu/g
Polony and Vienna	
TMA	<200 000 cfu/g
Coliform	<200 cfu/g
Yeast and mould	<1000 cfu/g
E. coli	0 cfu/g

CHAPTER 4

RESULTS

4.1 Introduction

This chapter presents results of the microbiological and chemical (phthalate) analysis on the food samples. Analyses were conducted in November 2010 and February 2011 and the chemical analysis was conducted from January 2009 to February 2011 on samples purchased at different times and randomly selected.

4.2 Microbiological analysis

The results shown for the different microbial growth is from the average of the number of colonies in the different dilution plates.

Table 4.1 Results of the microbiological analysis in collected food samples in Pretoria in 2010

Tests	**Polony**		**Cheese**		**Vienna**	
	North	West	North	West	North	West
	5 Nov 2010	15 Dec 2010	5 Nov 2010	15 Dec 2010	5 Nov 2010	15 Dec 2010
TMA	40	$>10^6$	180	$>10^6$	360000	$>10^6$
Coliforms	NG	$>10^6$	NG	NG	NG	NG
Salmonella	NG/25g	NG/25g	NG/25g	NG/25g	NG/25g	NG/25g
S. aureus	NG	NG	NG	NG	NG	NG
L. monocytogenes	NG/25g	NG/25g	NG/25g	NG/25g	NG/25g	NG/25g

Bacterial count in CFU/g; NG = No growth

Table 4.1 shows that none of the tested pathogens were identified in all the food samples that were tested. The same kinds of tests were repeated six times on different samples at different time frames and no pathogens were detected except for coliforms in polony which were found to be $>10^6$ cfu/g. Therefore, no further tests were conducted using the pathogens parameters. Yeast and mould, coliforms, TMA and *E. coli* where chosen as parameters

which would be tested for in the remaining food analysis. Yeasts and moulds are common in food spoilage.

Table 4.2 Determination of the presence of food pathogens and spoilers in vienna samples purchased in Pretoria in 2011

Tests	**North**		**South**			**East**			**West**
	2 Feb.	15 Feb.	2 Feb.	15 Feb.	21 Feb.	2 Feb.	15 Feb.	21 Feb.	21 Feb.
TMA $x10^3$	21300	5200	5300	34000	14100	103000	68	1380	32000
Coliforms $x10^3$	150	5.6	0.07	780	7	NG	0.02	4.8	2620
E. coli	NG	NG	NG	NG	NG	NG	NG	NG	NG
Yeast $x10^3$	NG	20	NG	14900	0.02	0.03	0.25	2.6	0.48
Mould	NG	NG	NG	NG	NG	0.06	NG	NG	NG

Bacterial count in CFU/g; NG = No growth

It can be seen from the above Table that TMA is always the highest as compared to the other tests followed by yeast, coliforms and then mould. No bacterial count was determined for *E. coli*. Higher numbers were observed in samples from Pretoria south.

Table 4.3 Determination of the presence of food pathogens and spoilers in polony samples purchased in Pretoria in 2011

Tests	North			South	East			West		
	2 Feb.	15 Feb.	21 Feb.	2 Feb.	2 Feb.	15 Feb.	21 Feb.	2 Feb.	15 Feb.	21 Feb.
TMA $x10^3$	3.8	0.41	15000	23000	73000	$3.1x10^4$	$4.9 x10^4$	-	-	$7.5 x10^4$
Coliforms $x10^3$	NG	NG	NG	NG	NG	20.4	5.5	-	-	NG
E. coli	NG	NG	NG	NG	NG	NG	NG	-	-	NG
Yeast $x10^3$	NG	NG	400	NG	1110	59	37	-	-	3500
Mould $x10^3$	NG	NG	NG	0.06	NG	0.09	NG	-	-	NG

- Bacterial count in CFU/g
- NG = No growth;
- - no test conducted due to financial constraints

It is clearly seen, from Table 4.3, that the samples which were tested for the new parameters showed some microbial and fungal activity. This is further discussed in the following chapter.

Table 4.4 Determination of the presence of food pathogens and spoilers in cheese samples purchased in Pretoria in 2011

Tests	North			South			East			West
	2 Feb.	15 Feb.	21 Feb.	2 Feb.	15 Feb.	21 Feb.	2 Feb.	15 Feb.	21 Feb.	21 Feb.
TMA $x10^3$	0.5	2.16	0.12	47000	11400	11500	103	18600	2610	71000
Coliforms $x10^3$	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
E. coli	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Yeast $x10^3$	0.01	NG	NG	NG	158	NG	82	NG	NG	8500
Mould $x10^3$	NG	NG	NG	NG	NG	NG	NG	NG	0.01	92

- Bacterial count in CFU/g
- NG ─ No growth

No growth was observed for coliforms and *E. coli*. There was little activity with mould and slightly more for yeast. The total microbial activity was the highest as it was expected.

Figure 4.2 shows the amount TMA in each sample type through a percentage representation in all four sites as observed. For North, South and East sites, data of samples analysed on 2 February was used to draw the graphs while for West site, samples from 21 February were used. This was mainly because for West site, as it can be seen from Table 4.2, 4.3 and 4.4, only samples on 21 February were analysed due to financial constraints. These dates were chosen in order to illustrate the trend observed in TMA across the different sites.

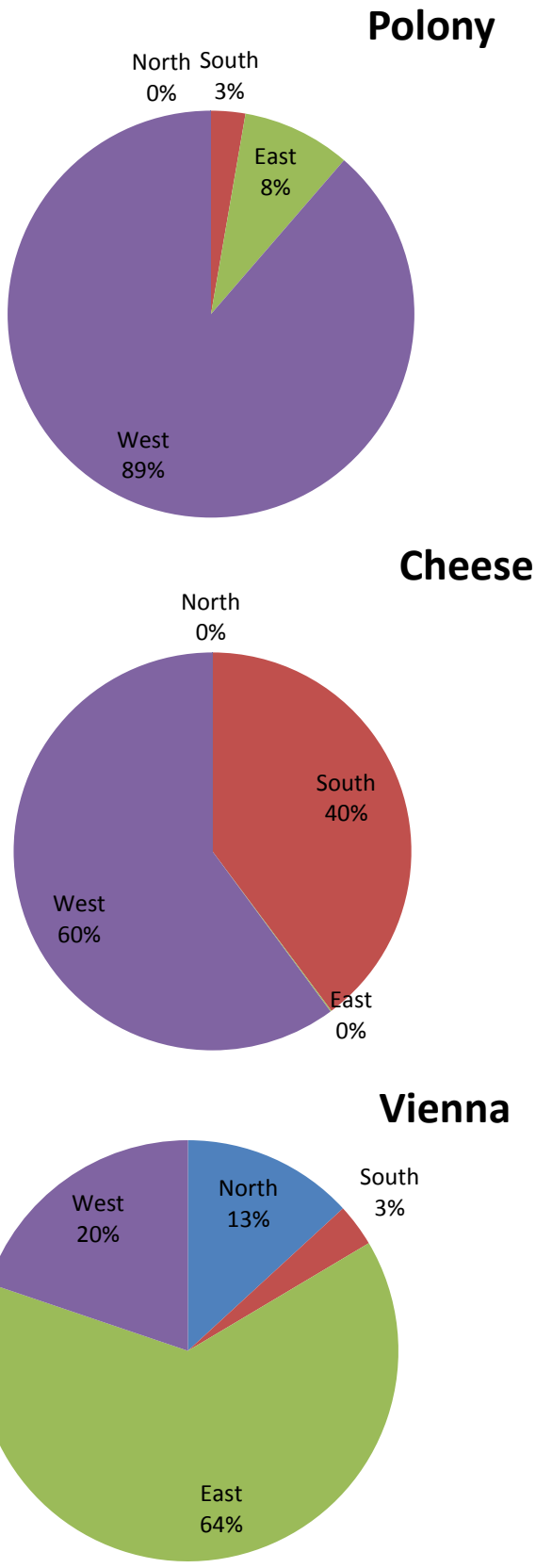

Figure 4.2 Pictorial comparison of TMA in three sample types showing the comparison of the parameters in each sample type

4.3 Chemical analysis

Cheese, polony and vienna samples were analysed for the presence of phthalate esters. A total of 114 food samples were analysed; 42 cheese samples, 39 polony samples and 33 vienna samples. All the samples were randomly collected (purchased) from the four Tshwane metropolitan regions.

4.3.1 Calibration and chromatograms

The food samples were extracted using Soxhlet extraction and analysed by GC. Calibration curves were prepared for each of the standard compounds. Each calibration curve was linear and the concentration of the calibration solutions ranged from 0.2-0.8 mg/l. An example of a calibration curve is illustrated in Figure 4.3 below where the peak area is plotted against the standard concentration.

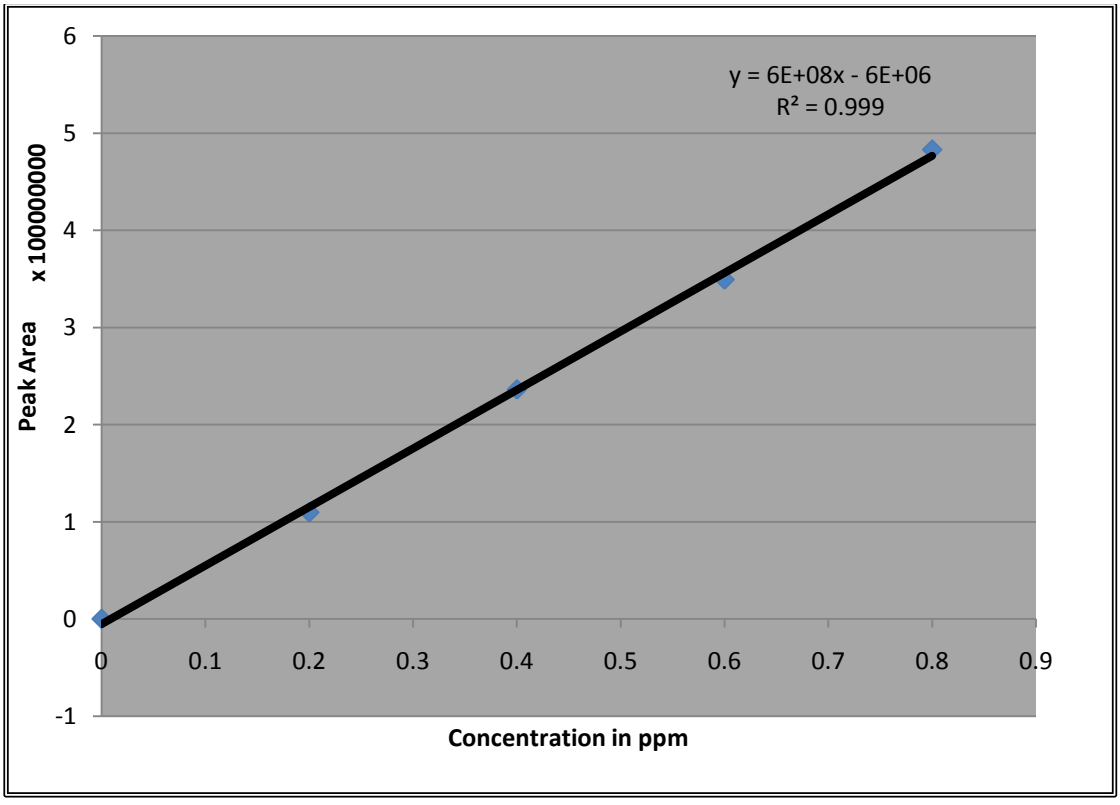

Figure 4.3 An example of a calibration curve prepared for Di-n-butyl phthalate (DnBP)

For all the sample concentration ranges that were determined, linear calibration graphs were obtained. The correlation coefficient ranged from

0.9921 to 0.999 where the average correlation coefficient is 0.9966. The curves were used to then determine the concentration of each phthalate ester found in the in food samples.

Table 4.5 Calibration data of the different phthalate

Compound names	coefficient (r^2)
DMP	0.9967
DBP	0.9974
DNBP	0.999
DEHA	0.9921
BBP	0.9979
Average correlation coefficient = 0.9966	

The elution of phthalates from the gas chromatographic columns was conducted in the order ISTD, DMP, DBP, DNBP, DEHA, and BBP. The retention times (minutes) for phthalate esters using the optimized GC conditions were ISTD- 6.445, DMP- 5.549, DBP- 7.353, DNBP- 7.699, DEHA- 8.921, and BBP- 8.953 as shown in Figure 4.4 below.

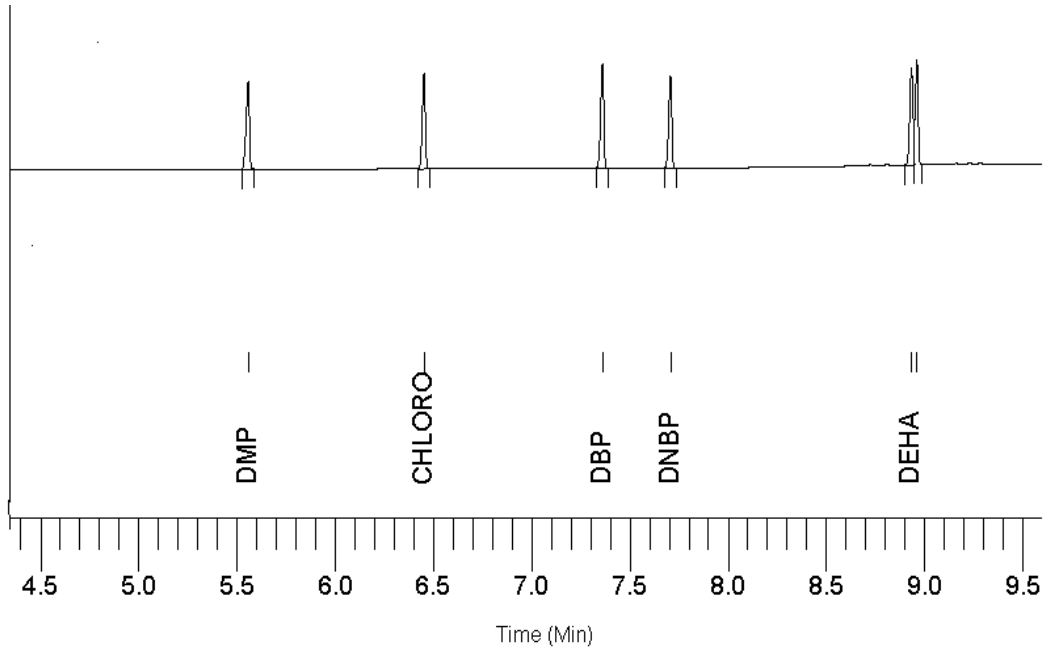

Figure 4.4 Gas chromatogram of the mixture of phthalate ester standards (ISTD-n chlorotetradecane, Dibutyl phthalate (DBP), Di-n-butyl phthalate (DNBP), Benzyl butyl phthalate (BBP), di-2-ethyl hexyl adipate (DEHA) and Dimethyl phthalate (DMP)

4.3.2 Sample concentrations

The blank determination with haxane gave a clean background with no contamination by phthalate esters. The concentrations of the detected phthalate esters ranged from 0.0333 to 4.7003 µg/kg. The dominant phthalate esters in all sites were DBP, DMP and BBP.

Table 4.6, 4.7 and 4.8 present the results of phthalate ester analysis of cheese, vienna and polony samples from Pretoria. The time frame ranged from March 2009 to February 2010. The common phthalate esters, which were detected, were DBP, BBP and DMP. In few samples, DNBP and DEHA were detected. Two to three different samples were analysed for phthalates on the same date, as it is observed in Tables 4.6, 4.7 to 4.8.

Table 4.6 Levels of phthalate esters (µg/kg) in cheese samples from Pretoria during the month of March, April, August, September 2009 and January, February and April 2010

Date	DBP	BBP	DMP	DNBP	DEHA
NORTH					
18 April 2009	bdl	bdl	bdl	bdl	bdl
12 August 2009	0.049	0.042	0.072	bdl	bdl
12 August 2009	0.054	0.050	0.086	bdl	bdl
10 September 2009	0.087	0.091	4.700	bdl	bdl
10 September 2009	bdl	0.420	bdl	0.925	0.120
10 September 2009	bdl	bdl	bdl	bdl	bdl
1 February 2010	bdl	0.094	bdl	bdl	bdl
SOUTH					
4 April 2009	bdl	bdl	bdl	bdl	bdl
12 August 2009	0.153	0.101	0.215	bdl	bdl
12 August 2009	0.071	0.039	0.083	bdl	bdl
8 September 2009	0.070	0.035	bdl	bdl	bdl
8 September 2009	bdl	bdl	bdl	bdl	bdl
8 September 2009	0.256	0.643	0.131	bdl	bdl
19 January 2010	bdl	0.067	0.072	bdl	bdl
20 January 2010	0.060	0.194	bdl	bdl	bdl
20 January 2010	0.055	0.053	bdl	bdl	bdl
21 January 2010	bdl	bdl	bdl	bdl	bdl
9 February 2010	bdl	bdl	bdl	bdl	bdl
4 April 2010	bdl	bdl	bdl	bdl	bdl
EAST					
31 March 2009	bdl	bdl	bdl	bdl	bdl
18 April 2009	0.081	0.123	0.161	bdl	bdl
12 August 2009	0.087	0.041	0.177	bdl	bdl
31 August 2009	0.092	0.673	bdl	bdl	bdl
31 Sep 2009	bdl	bdl	bdl	bdl	bdl
9 February 2010	bdl	bdl	bdl	bdl	bdl
WEST					
18 April 2009	bdl	bdl	bdl	bdl	bdl
27 Sep 2009	bdl	bdl	bdl	bdl	bdl
1 January 2010	bdl	bdl	bdl	0.090	bdl
1 February 2010	bdl	bdl	bdl	bdl	bdl
1 February 2010	bdl	bdl	bdl	bdl	bdl

bdl = below detection limit

Table 4.7 Levels of phthalate esters (µg/kg) in polony samples from Pretoria during the month of March, April, August, September 2009 and January to February 2010

NORTH					
Date	DBP	BBP	DMP	DNBP	DEHA
3 March 2009	0.110	0.039	0.147	bdl	bdl
18 April 2009	0.1100	0.041	bdl	bdl	bdl
20 April 2009	bdl	bdl	bdl	bdl	bdl
11 Aug 2009	0.042	bdl	0.118	bdl	bdl
13 Sep 2009	bdl	bdl	bdl	bdl	bdl
15 Aug 2009	bdl	bdl	bdl	bdl	0.075
19 Jan 2010	bdl	bdl	bdl	bdl	bdl
2 Feb 2010	bdl	bdl	bdl	bdl	bdl
SOUTH					
13 August 2009	0.064	0.040	0.080	bdl	bdl
13 August 2009	0.611	0.049	0.077	bdl	bdl
13 August 2009	0.129	0.061	0.056	bdl	bdl
13 September 2009	bdl	bdl	bdl	bdl	bdl
19 January 2010	bdl	bdl	bdl	bdl	bdl
21 January 2010	bdl	bdl	bdl	bdl	bdl
21 January 2010	bdl	bdl	bdl	bdl	bdl
27 February 2010	bdl	bdl	bdl	bdl	bdl
EAST					
3 March 2009	0.314	0.344	0.241	bdl	bdl
18 April 2009	bdl	bdl	bdl	bdl	bdl
20 April 2009	0.052	bdl	0.149	bdl	bdl
12 Aug 2009	0.095	0.036	0.126	bdl	bdl
4 Sep 2009	0.063	0.064	bdl	bdl	bdl
4 Sep 2009	0.076	0.079	bdl	bdl	bdl
4 Sep 2009	bdl	0.036	bdl	bdl	bdl
2 Feb 2010	bdl	bdl	bdl	bdl	bdl
2 Feb 2010	bdl	bdl	bdl	bdl	Bdl
WEST					
18 April 2009	bdl	bdl	bdl	bdl	bdl
20 April 2009	bdl	bdl	bdl	bdl	bdl
12 Aug 2009	0.076	0.067	bdl	bdl	bdl
13 September 2009	bdl	bdl	bdl	bdl	bdl
2 Feb 2010	bdl	bdl	bdl	bdl	bdl
2 Feb 2010	bdl	bdl	bdl	bdl	bdl

bdl = below detection limit

Table 4.8 Levels of phthalate esters (µg/kg) in vienna samples from Pretoria during the month of March, April, August, September 2009 and January to February 2010

Date	DBP	BBP	DMP	DNBP	DEHA
NORTH					
3 March 2009	0.078	0.033	0.816	bdl	bdl
20 April 2009	0.209	0.177	0.169	0.047	bdl
12 August 2009	0.092	0.673	bdl	bdl	bdl
27 Sep 2009	bdl	bdl	bdl	bdl	bdl
3 February 2010	bdl	bdl	0.092	bdl	bdl
SOUTH					
12 August 2009	0.133	0.035	0.084	0.119	bdl
12 Sep 2009	bdl	bdl	bdl	bdl	bdl
20 January 2010	bdl	bdl	bdl	bdl	bdl
21 January 2010	bdl	bdl	bdl	bdl	bdl
EAST					
3 March 2009	0.124	0.096	0.200	0.071	bdl
20 April 2009	bdl	bdl	bdl	bdl	bdl
4 Sep 2009	0.074	0.069	bdl	bdl	bdl
7 Sep 2009	bdl	0.075	bdl	bdl	bdl
8 Sep 2009	bdl	bdl	bdl	bdl	bdl
9 Sep 2009	bdl	0.031	bdl	bdl	bdl
WEST					
3 March 2009	0.081	0.048	bdl	bdl	bdl
20 April 2009	0.081	0.086	0.185	bdl	bdl
20 April 2009	bdl	bdl	bdl	bdl	bdl
20 Sep 2009	bdl	bdl	bdl	bdl	bdl
3 Feb 2010	bdl	bdl	bdl	bdl	bdl
3 February 2010	bdl	bdl	bdl	bdl	bdl

bdl = below detection limit

A monthly comparison of phthalate detection in cheese is illustrated graphically in Figures 4.5 a-d. For the months of August and September, the first result in each site was chosen. An average could not be calculated since multiple results of one date represented the number of samples analysed on the specific date.

Figure 4.5a Comparison of the levels of phthalate esters in cheese samples in the month of April, 2009

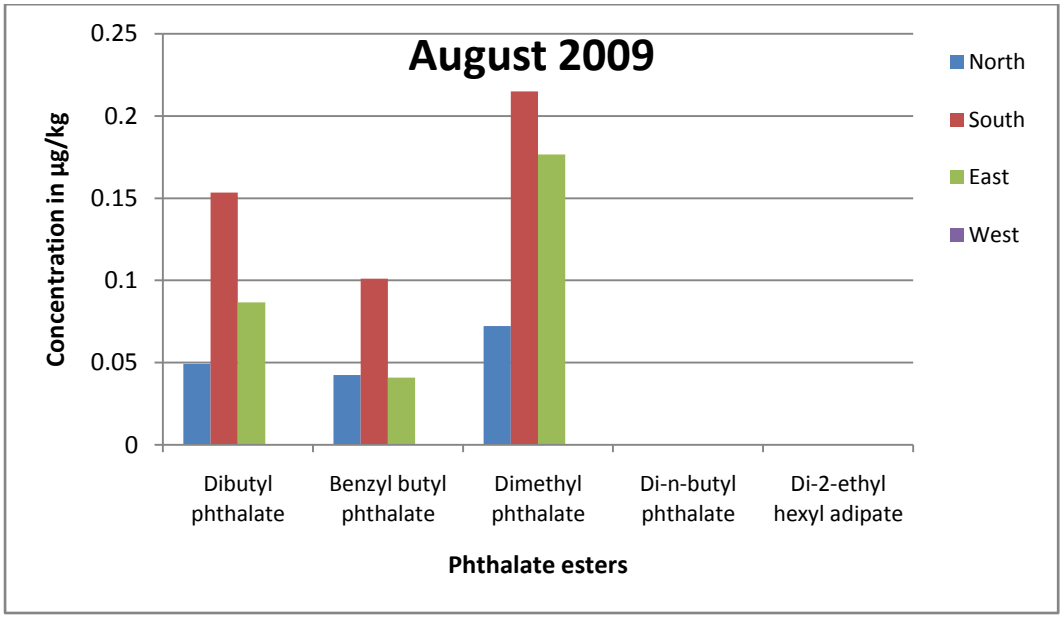

Figure 4.5b Comparison of the levels of phthalate esters in cheese samples in the month of August, 2009

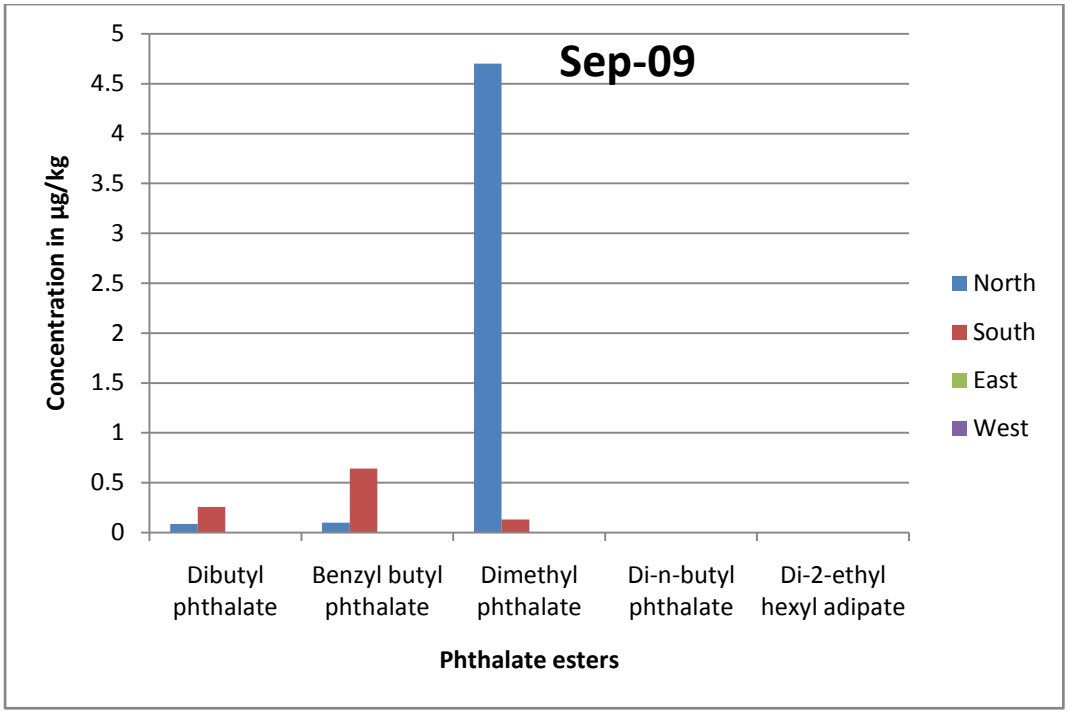

Figure 4.5c Comparison of the levels of phthalate esters in cheese samples in the month of September, 2009

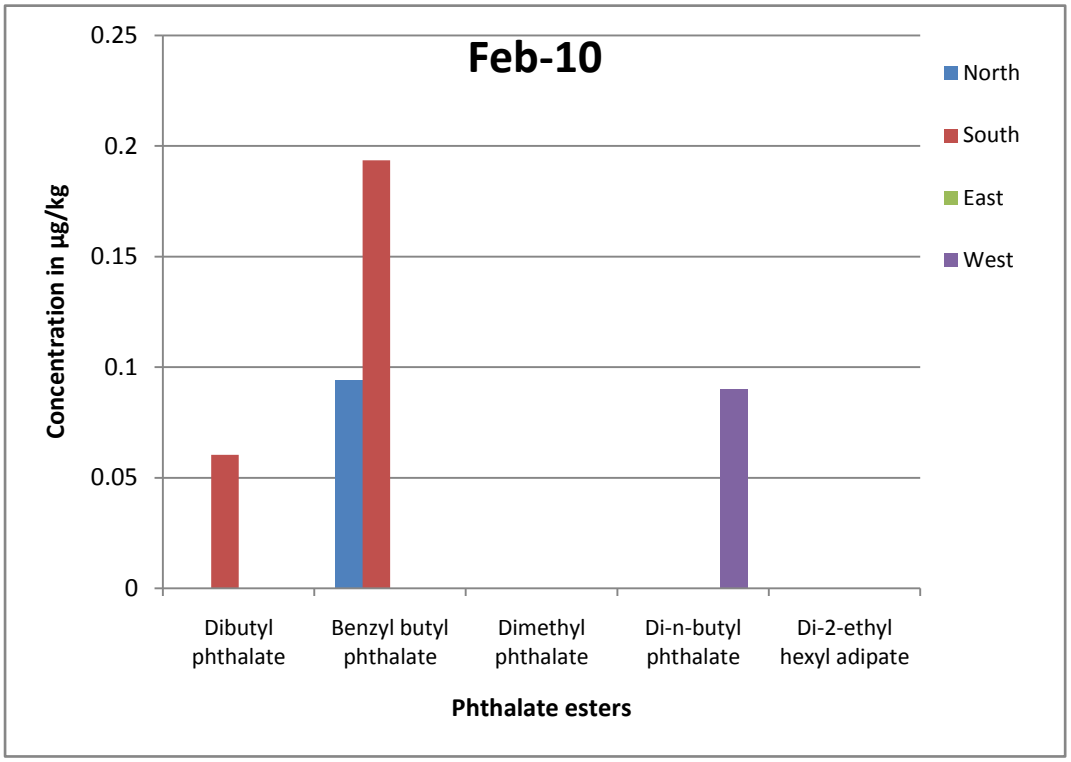

Figure 4.5d Comparison of the levels of phthalate esters in cheese samples in the month of February, 2010

Figures 4.5 a-d showed a graphical comparison of monthly distribution of phthalate esters in cheese samples. The presence of phthalates in April 2009, August 2009, September 2009 and February 2010 is as shown.

Table 4.9 Percentage recoveries of phthalate ester from spiked food samples

Phthalate esters	**Cheese**	**Vienna**	**Polony**
1-chlorotetradecane(ISTD)	115	88	45
	78	110	33
	74		
Average % recovery (ISTD)	**89 ± 22.6**	**99 ± 15.6**	**39 ± 8.5**
Dibutyl phthalate, DBP	105	92	35
	89	106	34
	86		
Average % recovery (DBP)	**93.3 ± 10.2**	**99 ± 9.9**	**34.5 ± 0.7**
Di-n-butyl phthalate, DNBP	102	90	50
	89	104	40
	86		
Average % recovery(DNBP)	**92.3 ± 8.5**	**97 ± 9.9**	**45 ± 7.1**
Benzyl butyl phthalate, BBP	123	113	33
	92	98	37
	88		
Average % recovery (BBP)	**101 ± 19.2**	**105.5 ± 10.6**	**35 ± 2.8**
Di-2-ethyl hexyl adipate, DEHA	47	86	41
	89	51	32
	85.6		
Average % recovery (DEHA)	**73.9 ± 23.3**	**68.5 ± 24.7**	**36.5 ± 6.4**
Dimethyl phthalate, DMP	75	80	36
	74	103	32
	71		
Average % recovery (DMP)	**73.3 ± 2.1**	**91.5 ± 16.3**	**34 ± 2.8**

The percentage recoveries for cheese and vienna samples was satisfactory whereas that of polony samples was below the acceptable percentage which is 60%.

4.3.3 Correlation coefficient

A monthly correlation coefficient of samples was determined. Through the results, the relationship between the samples was identified.

Table 4.10 a-h Monthly correlation coefficient of cheese, polony and vienna samples

a) Correlation coefficient of phthalates DBP and BBP for Cheese North samples

Sample size	5
Correlation coefficient r	**-0.5784**
Significance level	P=0.3070
95% Confidence interval for r	-0.9671 to 0.6205

b) Correlation coefficient of phthalates DBP and BBP for Cheese South samples

Sample size	6
Correlation coefficient r	**0.8702**
Significance level	P=0.0242
95% Confidence interval for r	0.1995 to 0.9857

c) Correlation coefficient of phthalates DBP and BBP for Cheese East samples

Sample size	4
Correlation coefficient r	**0.6737**
Significance level	P=0.3263
95% Confidence interval for r	-0.8153 to 0.9923

d) Correlation coefficient of phthalates DBP and BBP for Polony North samples

Sample size	3
Correlation coefficient r	**0.9991**
Significance level	P=0.0275

e) Correlation coefficient of phthalates DBP and BBP for Polony South samples

Sample size	3
Correlation coefficient r	**0.02668**
Significance level	P=0.9830

f) Correlation coefficient of phthalates DBP and BBP for Polony East samples

Sample size	5
Correlation coefficient r	**0.9591**
Significance level	P=0.0099
95% Confidence interval for r	0.4991 to 0.9974

g) Correlation coefficient of phthalates DBP and BBP for Vienna North samples

Sample size	3
Correlation coefficient r	**-0.2085**
Significance level	P=0.8663

h) Correlation coefficient of phthalates DBP and BBP for Vienna East samples

Sample size	6
Correlation coefficient r	**0.7390**
Significance level	P=0.0933
95% Confidence interval for r	-0.1813 to 0.9693

In conclusion, all results presented in this chapter will be further discussed in chapter 5, which follows. The significance of each result will be highlighted and compared to previous studies, which have been conducted in the same area. Conclusions and recommendations will also be highlighted.

CHAPTER 5

DISCUSSION

5.1 Introduction

This section discusses the findings or results at length with a focus on the trends identified and their implications.

5.2 Determination of food pathogens and spoilers in food sample

Safety of foodstuffs is critical in South Africa as well as other countries. According to WHO, many governments in different countries are striving to intensify their efforts to improve the security of foods (WHO, 1997). Hence, there are regulations that are set for ensuring food safety. In South Africa, foodstuffs which are produced, processed, and sold, including those which are imported from other countries, are all governed by the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) (FCD) from a human health perspective. This Act aims to "control the sale, manufacture importation and exportation of foodstuffs, cosmetics and disinfectants; and to provide for incidental matters" (South African FCD Act, 1972). The Directorate: Food Control at national level administers this act. The mission of this Directorate, is to "ensure an optimal non-personal preventative primary health care service in respect of the safety of food for the South African community, based on basic needs and the right to make informed choices without being misled by means of scientifically founded legislation, auditing and information actions" (Directorate: Food Control, (n.d.)). One prominent regulation stipulated is that foodstuffs should not contain microorganisms at levels which may cause harm to humans upon consumption.

In the South African Health Act, 1977 (Act no. 63 of 1977), there are regulations regarding the premises in which food handling, processing, production, manufacturing, packing, storing and preparation occur. There are also standards and requirements of apparatus and equipment used in food handling. The

regulation of the manner of transportation of different foodstuffs also exists; which stipulates the nature of the holders used for food storage. It is emphasised that the food should be handled hygienically and protected against any form of contamination that can lead to health hazard.

5.3 Analysis of pathogens in test samples

Salmonella is usually implicated in raw foods of animal origin such as chicken, poultry products, which includes eggs, and turkey (Yousef and Carlstrom, 2003). In this study, *Salmonella* was tested (Table 3.1) because it is a common-food pathogen causing a disease known as salmonellosis. Workers who work in food harvesting industries, processing and service are suspected to not observe appropriate personal hygiene and hence can be potential sources of food contamination with *Salmonellae*. However, it is not all *Salmonella* serovars known to be pathogenic to humans but all *Salmonellae* species are unacceptable in ready-to-eat foods (Food and Environmental Hygiene Departments, 2002; Department of Health Directorate, n.d.).

From the results obtained (see Table 4.1), there was no identification of any of the common-food pathogens (*Salmonella, S. aureus* and *L. monocytogenes*) in all the samples analysed. It can then be concluded (since the test was repeated six times and still yielded same result) that the food samples were all free from common-food pathogens. This does not, however, suggest that the food is entirely safe for consumption. This is because Gilbert *et al*. (2000) stated that the presence of salmonella and other pathogens in ready-to-eat foods may not necessarily cause disease, however, there has been microbiological and epidemiological evidence that a small number of these food pathogens have caused illness.

According to the Food and Agriculture Organisation of the United States, the limit for *Salmonella*, *S. aureus* and *L. monocytogenes* in cheese is 0/ml, 0/ml and 0/ml respectively (Codex Alimentarius Commission, 2002). This means that none of the above mentioned pathogens should be found in cheese. These limits correlated well with the results obtained in this study since not any of these pathogens were detected in the cheese. The limit for *Salmonell*a and *S. aureus* in cooked sausages was set at 0/ml and 10^4/ml respectively. No limit was reported for *L.*

monocytogenes. Nevertheless, Yousef and Carlstrom (2003) stated that in the detection of *L. monocytogenes*, since the food sample weighing 25g is analysed, the theoretical minimum detection limit of the analysed sample is one *Listeria* per 25g of food sample. This is because when *Listeria* is present in food, it is usually in extremely low counts; therefore, direct plating is usually not the best detection method to be used. According to Yousef and Carlstrom (2003), the minimum detection limit is also remarkably low because, even the smallest amount of *L. monocytogenes* can be considered an infectious dose. In support of this, Gilbert *et al*. (2000) indicated that indeed some quality standards require zero level of *L. monocytogenes* at the production stage. This would mean that any amount of *L. monocytogenes*; e.g. 10^3 CFU/g; found in ready-to-eat food will be representing a potential health risk.

In Table 4.1, results obtained showed that none of the three pathogens tested for were detected. This then resulted in the changing of the parameters used, and different tests for food pathogens and spoilers introduced. Yeast and mould, coliforms, TMA (total microbial count) and *E. coli* were chosen as parameters in the remaining food analysis.

In food microbiological analysis, tests for indicator organisms such as enteric indicator bacteria and aerobic mesophilic plate counts are done in order to demonstrate the presence of pathogens in the tested food (Department of Health Directorate, (n.d.)). The indicator microorganisms tested for in the present study, were, coliforms; *E. coli*; yeast and mould; and total microbial activity (TMA). These are tests stipulated as acceptable by the South African Department of Health under the Directorate: Food Control and recognised internationally, as well.

From the results obtained in the current study, in Table 4.2, high levels of viable bacteria were detected in vienna samples. The TMA ranged from 68000 to 103 000 000cfu/g. According to the South African Department of Health, the proposed microbiological parameters to be used as guidelines for processed meats (vienna, polony, etc.) are stipulated as follows: the TMA should not be greater than 200 000cfu/g. From the results obtained for vienna, only one sample from Pretoria east complied with the limit. The rest of the samples were over the allowed number of viable bacteria. This then means that the food samples found with large

populations of microorganisms can be regarded as unwholesome as stipulated by the Department of Health in its regulation. The microorganisms detected do not necessarily have to be pathogenic, but if the numbers found are unacceptable according to the regulation, the foodstuff can then be regarded as not safe for human consumption.

One of the observations when the samples were purchased, was that most of the foodstuffs did not have any expiry dates indicated, and the storage temperature was also too high, i.e. some of the products were stored in temperatures between 10-25°C which is inappropriate for processed meats. This then could be reason to justify the results obtained. Microorganisms such as *S. aureus* and *Salmonellae* grow rapidly at temperature between 5-60°C. The high numbers detected can be due to contamination of the raw materials during preparation and can also indicate unacceptable temperature storage conditions.

In the UK, the expert panel on microbiological safety of food of the Food and Environmental Hygiene Departments impose some regulations for the acceptable numbers of microorganisms in ready to eat foods. For polony and vienna, the TMA acceptable limit is not greater than 10^7 cfu/g (Food and Environmental Hygiene Departments, 2002). The results obtained in this study also do not comply with those of the UK as from Table 4.2 and Table 4.3, the TMA for Vienna and polony was as high as 2.13 x10^7 cfu/g and 4.900000 x10^9 cfu/g respectively.

Table 4.2 shows the presence of yeast and mould in vienna samples from the south, east and west. Highest numbers of yeast were 1.49 x 10^7cfu/g, found in one sample from Pretoria south. The Department of Health states that consumers should not eat food that is visibly mouldy. When the samples were purchased and taken for testing, there was no visibility of mould. From the results, the yeast numbers were high; however, it is inconclusive to declare any indication of human health hazard.

The presence of high numbers of mould and yeasts in food indicates that there was poor sanitation and handling (Department of Health, n.d.). The wrong storage temperatures could have contributed to development of yeast and mould. Another reason for development of yeast and mould could be due to inadequate processing

or even post process contamination as was the case in a study by Raji and den Aantrekker (2005). It was demonstrated through a review of many outbreaks that the presence of vegetative pathogens such as *Salmonella* spp. or *Listeria monocytogenes* in the consumed products was frequently due to post-process recontamination. From the samples tested, the numbers of yeast and mould were high. This indicates that there was indeed contamination of the food samples. The food samples analysed were purchased in small departmental stores around Pretoria. Most of the small departmental stores do not comply with proper regulations set by the Department of Health when it comes to food handling as was the case in this study. One other aspect which is not adhered to is the indication of the best-before-date or date of expiry on the packaging of the food. One of the differences between small departmental stores and the big departmental stores in South Africa is that the big stores adhere to expiry dates. Once the expiry date arrives, the big departmental stores discard all the foodstuffs which are on the shelf, primarily in order to protect consumers, even if there is no apparent spoilage. This is, however, remarkably different from what happens in the smaller departmental stores. Most of the samples purchased and analysed for the presence of microorganisms had no expiry dates. This would then mean that, the food can be unsafe and yet can still be sold only because there is no visibility of spoilage. Hence the presence of some food spoilers obtained in this study would serve to indicate the negligence that is in some of the smaller food stores.

Yousef and Carlstrom (2003) mentioned that yeasts and moulds are classified under fungi. When these fungi are found in acidic food, they alter the pH of food by consuming all the acidic ingredients and as a result, they raise the pH. It is also a requirement that the food industry should prevent the growth of hazardous bacteria in food by using different methods and parameters. Once the fungi have altered the pH of the food, the conditions of the found change, and hence, will support the growth of hazardous bacteria, which were initially inhibited by the acidity of the food. This would also be the same if the food is stored under inappropriate temperature in order to prevent the growth of certain microorganisms.

The highest number of coliforms found in vienna (Table 4.2) was 2.62×10^6 cfu/g and 2.04×10^4 cfu/g for polony samples (Table 4.3). The limit set by the

Department of Health is not greater than 200 cfu/g. It can be seen that the numbers found in the tested samples were significantly greater than the acceptable limit. It was, therefore, necessary to further study the samples to determine the presence of *E. coli* in the number of coliforms detected. For both polony and vienna samples, no *E. coli* were present in all samples.

The presence of coliforms in high numbers, in vienna and polony, showed that there was poor sanitation, poor hygiene handling or even inadequate storage (Department of Health Directorate, (n.d.)). High levels of yeast were found in polony samples; ranging between 3.7×10^4 and 3.5×10^6 cfu/g. This could also be attributed to storage in inappropriate temperatures. Prior and Casaleggio (1978) studied the microbiology of polony by microbiogically analysing 25 polony samples. It was found that yeast and moulds developed after incubation at 5°C for 12 days with yeast dominating.

The regulation set for cheese by the Department of Health for coliforms is 1000 cfu/g and *E. coli* at 0 cfu/g. For all cheese samples (Table 4.4) from all the sites, no coliforms and *E. coli* were detected. This shows that there was not any suspicion of faecal contamination. However, the TMA numbers ranged from as low as 1.2×10^2 cfu/g to as high as 7.1×10^7 cfu/g. This is, however, expected for cheese since it is a dairy product and lactic acid bacterial culture are used in the fermentation process.

The poor microbiological quality of these foods is primarily associated with storage of foodstuffs in temperatures above 6°C. The food handlers could have also not been hygienically fit to handle food, hence the development of microbes higher than the acceptable limits. This is because some of the samples purchased were most probable handled since there were cut and packaged into small quantities.

In the four metropolitan regions of Pretoria, pathogens i.e. coliforms in polony samples from all sites were detected in the food samples. This shows that there was a certain level of contamination in the foodstuffs. Furthermore, Figure 4.2 shows that, in polony samples TMA was about 89% in PTA west, which was higher than cheese (60%) and in vienna 20% comparatively. These results only represent the level of microorganisms at the time that the tests were conducted, but do not

represent the overall level of contamination in each site. Vienna samples showed high microbial activity in PTA east samples.

The overall trends, identified through the results, showed that Pretoria west had higher microbial activity as high as 7 x 10^8cfu/g as compared to other sites. However, yeast and coliforms dominated all the sites in all the three samples tested. This can be seen in Tables 4.2, 4.3 and 4.4. WHO (1995), indicated in a survey performed in Europe, that almost 25% of the food-borne outbreaks could be traced back to recontamination. Other contributing factors to the presence of pathogens in prepared foods are insufficient hygiene (1.6%), cross-contamination (3.6%), processing or storage in inadequate rooms (4.2%), contaminated equipment (5.7%) and contamination by personnel (9.2%). These reasons to contamination can also be adopted for the present study since it has been highlighted that most of the samples were stored under inappropriate temperature, which then encouraged, the development of microorganisms.

As highlighted by Ray (2001) that many processed foods typically contain several types of moulds, yeasts and bacteria that have the potential to multiply and cause spoilage, it has certainly been confirmed in the present study. The implication of the presence of high microbial activity which does not comply with the limits set by the Department of Health could be threatening to human health after consumption. Depending on the microorganism found in each food sample, health problems, which could be a threat to human health, includes food-borne illnesses, which are caused, by ingestion of contaminated food.

5.4 Calibration of GC and Chromatograms of extracted food samples

Extraction of plasticiser by Soxhlet extraction is a time-consuming process, which is widely preferred. However, in most literature, this method has been replaced by infusion in chloroform since it is more rapid and quantitative for thin films (Mercer, 1990). Chapter 2 (Literature review) gives a detailed discussion as to why Soxhlet extraction was the method of choice in the current study.

The GC-FID analysis was chosen in this study since it permits a wide range of plasticisers to be identified. The internal standard used was 1-chlorotetradecane;

since it was found to be similar in nature to the plasticisers under study. There are a number of methods used for the clean-up of phthalate esters prior to analysis by gas chromatography. This study used the florisil column for the clean-up.

The GC-FID was used in the study because it has a number of advantages over the GC-MS. Phthalates are classified as organic compounds, as a result, GC-FID is preferred in the analysis of organic compounds because it has high sensitivity and low noise. Another advantage is that the response of the detector is not affected by changes in the mobile phase flow rate because FID is mass sensitive rather than concentration sensitive.

The GC-FID chromatogram of contaminated-food samples showed smooth, clean and symmetrical peaks for all the phthalate esters, which were detected.

5.5 Comparison of the level of phthalate esters detected in cheese, polony and vienna

Phthalates were detected at various levels in all food sample types spread out from the different sites, i.e. cheese, vienna, and polony samples. Tables 4.6, 4.7 and 4.8 show phthalate levels in three sample types from Pretoria North, South, East and West. For all samples analysed, it was not indicated on the packaging when the samples where produced. As a result, the probability of buying the samples produced in the same months cannot be ruled out; this is more likely for the samples analysed in March and April 2009; August and September 2009; January and February 2010. This is because the months come one after the other. Therefore, the possibility of sample purchased in August 2009 to still be in the shop in September 2009 is high.

In cheese samples analysed (see Table 4.5) from March 2009 to April 2010, the level of DBP contamination ranged from 0.049 (August 2009) to 0.673 µg/kg (August 2009), which as compared to published results, is lower than the limit. Samples analysed in 2010 in the months January, February and March showed lower level of PEs as compared to samples analysed in 2009 (March, April, August and September). In a reported study, the level of DBP in cheese was found to be greater than that in paper and board packaging (MAFF, 1995), at an exceptionally

low level of about 10 mg/kg. The study was conducted in the UK, and the result obtained did not however, conclude that DBP cannot be found in plastic packaging. The present study however showed the detection of DBP in plastic packaging as it was able to migrate from the packaging to the food samples tested for.

In polony samples (see Table 4.6), DBP levels ranged from 0.0412 in April 2009; which was the lowest DBP level detected; to 0.611µg/kg in August 2009; which was the highest DBP level detected. This was even lower levels as compared to those found in cheese samples. In polony samples tested in the year 2010 in January and February, no PEs were detected.

Even lower levels of DBP were detected in vienna samples ranging from 0.074 to 0.209 µg/kg in September 2009 and April 2009 respectively.

Trace levels of DBP can migrate from the packaging material into the food samples (MAFF, 1995). The concentration found in the food samples however, may not solely be as a result of migration of the phthalate from the packaging material. It may also be attributed to the nature of the environment where food processing and packaging occurs. Evidence of this was shown in a study by Sharman *et al*. (1994), which confirmed that DEHP contamination in cheese, milk, and cream, did not only come from the packaging, but from the overall environmental contamination. The same argument can also be adopted for the presence of DBP in cheese, polony and vienna samples, that since not much literature shows the presence of DBP in plastic packaging, then its contamination in cheese, although in low levels, could have been from widespread environmental contamination.

From the tested samples, another phthalate detected was BBP. As shown in Table 4.6, in cheese samples, the levels ranged from 0.0001 to 0.673 µg/kg. According to the Scottish Environment Protection Agency (SEPA, n.d.), the phthalate BBP is commonly found in an industry producing it and using it in a variety of products. This phthalate can then be released from landfills to soils and trace amounts of it into the air. The route of exposure to man, can occur through its ability to survive long in the environment, which can then lead to its accumulation by different plants, leading to animals being exposed. This can also explain its presence in the

analysed food samples. Since trace amounts of this phthalate were detectable in analysed foods, ingestion of the contaminated food can lead to human exposure.

In literature, the documented BBP human health effects include irritation of the oesophagus, irritation of the respiratory tract, and skin irritation. SEPA reported that there is only slight evidence of the full effects of BBP on human health. Hence the International Agency for Research on Cancer recorded BBP as not classifiable as to its carcinogenicity to humans, and as a result, it was concluded that normal background levels BBP cannot be considered as having any adverse effect on human health (SEPA, n.d).

According to Goulas *et al.* (2000), the fat content in the food samples has an impact on how much phthalate esters can migrate into the foodstuffs. The study focused on the migration of DEHA into three different types of cheese. The cheese containing more fat content had more DEHA after exposure. This might also explain the result obtained in the analysis of DEHA in cheese, polony and vienna. From table 4.6, 0.120 µg/kg DEHA was found in cheese sample as compared to vienna, which was below detectable limit, and polony which had 0.075 µg/kg.

In cheese samples, all the samples purchased in April 2009 tested negative for all the five phthalates. However, in the north site, August 2009 showed to have the highest levels of phthalates as compared to September 2009. There could be different reasons for this trend. One of the possibilities could be the duration the food sample was in contact with the plastic packaging. The longer the contact time, the higher the level of phthalate ester migrating into food sample (Brown and William, 2003). As it has already been indicated, samples purchased had no expiry dates indicated, this may lead to the possibility that they remain on the shelf for longer periods i.e. samples purchased and analysed in March 2009 could have still been in the shelf in April 2009. If this is the case, then samples purchased and analysed in August 2009 were covered with plastic for longer than those tested in September 2009.

In a study conducted by Goulas *et al.* (2000), the migration of DEHA in cheese samples was studied with a focus on the contact time. In Kafalotyri cheese, it was observed that, at 1h contact time, 81.1 mg/kg DEHA was detected; at 3h, 164

mg/kg, and at 10h, 201 mg/kg detected. The trend observed was that the longer the contact time between the plastic wrapper and food, the higher the phthalate migrates into the food sample, resulting in high amount detected during analysis.

Even though there was phthalates detected in the samples tested, most of the parameter relevant to the migration process was unknown. This parameter would include the history of the samples before they were purchased, the environment of the processing and packaging site, and the hygiene of the sample handlers.

Only three phthalate esters were detected in cheese samples from Pretoria East out of the five tested which were used as standards; these where DBP, BBP and DMP. In the month of April 2009, Pretoria East site showed dominance in the presence of the DBP, BBP and DMP while, in the other sites, there was no detection of any of the phthalates. The graphical representation of the result in Figure 4.5 a-d illustrates this well; showing the monthly comparison of phthalate esters in cheese samples. However, as compared to August 2009, DBP, BBP and DMP were detected in Pretoria North, South and East. In the months of September 2009 and February 2010, different phthalates were detected. The phthalates DMP, dominated in the north site in September 2009, while BBP dominated the south site in February 2010.

The trend observed from the results showed that as different samples are tested, in different sites, the amount of the phthalates which migrate into the food sample will vary. This would then mean that, the extent of exposure to humans will be determined by the amount of phthalate detected in the sample at the time of consumption. Two individuals can consume the same kind of food, but the level of exposure will be different. This depends on the amount of phthalate which has migrated from the plastic packaging into the foodstuff.

Percentage recoveries of phthalate esters from the spiked samples were detected as it can be seen in Table 4.9. This is a technique used to validate the method of extraction. The percentage recoveries for cheese and vienna samples were satisfactory and ranged from 73-101% and 68-105% respectively; whereas those of polony samples were below the acceptable percentage which is 60%. This could be attributed to ineffective spiking. As explained in section 3.7.1, food samples

were spiked with 1m of 1mg/l. Polony samples were also spiked with same amount but the percentage recovery was very low. The nature of the sample could have also contributed to the low recovery.

5.6 Correlation coefficient

The correlation coefficient was determined in order to establish if the samples analysed were from the same source i.e., same shop. A strong positive correlation was found for cheese samples from the south (Table 4.10b), with an r = 0.8702. Polony samples from north (Table 4.10d) and east (table 4.10f) showed strong positive correlation at r = 0.9991 and r = 0.9591 respectively. It can therefore be concluded that the strong positive correlation is a direct representation that samples are from the same source. No significant relationship was found in Cheese North, Cheese East, Polony South, Vienna North and Vienna East samples.

The limits for TDI set by the United Kingdom Ministry of Fisheries and Food (MAFF) were exceeded. The TDI limit is set as follows: BBP at 0.1 µg/kg body weight/day; DBP at 0.05 mg/kg body weight/day (MAFF, 1996). This shows that it is essential for the packaging industry to make certain changes in the packaging material they use. This is important, since the type of food samples tested in the present study, are consumed almost in each South African household on a daily basis. Hence the recommendations are stipulated with the hope that it will have implications in the assessments to be made for future policy making in food industries across the country.

5.7 Conclusion

The presence of phthalates in analysed samples revealed the migration of phthalates from the plastic wrappers used to wrap each sample into the food samples. The detection of microorganism in food samples can be attributed to food that do not have expiry dates i.e. without best before date and were still kept on the shelf, although refrigerated to be purchased by consumers.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Introduction

The introduction and use of plastic represent one of the most relevant scientific progresses in the last century as discussed in detail in Chapter 1 of the present study. There had been growing concern over the past decades on the use of plasticized materials for the preservation and presentation of food materials to consumers. The use of these plasticized materials as food wrappers has been found to expose consumers to possible adverse health issues. However, suggestive human exposure data stir a serious concern that phthalate exposure may be detrimental to human health; particularly to fertility and reproduction. Report of toxicity and effect of phthalate esters in humans has been comprehensively discussed in Chapter 2.

It was therefore essential to conduct a thorough study on the incidence of PEs in cheese, polony and vienna as these food samples can be classified under foodstuffs used regularly in South African households. It was further of interest to investigate the development of microbial entities in the food samples and establish a relationship between the presence of PEs and development of microbes.

This final chapter answers the research problem, accepts or rejects the hypothesis, and concludes the findings and finally presents recommendations for future work.

6.2 Summary of the study

6.2.1 Microbiological study

a) Conclusion remarks

The results of the microbiological study provided details of the food pathogens; *Salmonella, S. aureus, E. coli* and *L. monocytogenes*; food spoilers; coliforms, yeast and mould; and overall microbial activity (TMA) in the tested samples during 2010-2011. The initial parameters tested: *Salmonella, S. aureus* and *L. monocytogenes* were not detected in any of the tested samples. However, coliforms were the most common in the samples, followed by yeast, mould and lastly no detection of any *E. coli*. The total microbial count (TMA) ranged from 6.8 x 10^4 to 1.03 x 10^8 cfu/g; coliforms ranged from no growth to 2.62 x 10^6 cfu/g; yeast ranged from no growth to 1.49 x 10^7 cfu/g; and mould ranged from no growth to 9.2 x 10^4 cfu/g. The results revealed that microbial activity was high in each sample type and showed the absence of pathogens. However, high levels of coliforms were detected, followed by yeast, and extremely low levels of mould. It can then be concluded that the presence of microorganisms in the food samples is due to poor sanitation, poor hygiene handling or even inadequate storage in inappropriate temperatures.

In Chapter 1, section 1.7; Figure 1.2 shows the common sources of food contamination. In the present study, the source which could be suggested to have promoted the development of the microorganisms found in each sample is the packaging material; this is because with some of the food samples stored at inappropriate temperatures, the development of microbes might have been encouraged.

The absence of the expiration dates contributed to the development of the microorganisms. Hence it is recommended that, through the appointment of health inspectors, regular assessment in the departmental stores can be done. This will also help to monitor and ensure that the expiration dates are clearly marked on each sample and that the expired foods will be discarded appropriately.

b) Future research

Future research into different/other types of food samples such as meat, bread and tests for more microorganisms such as *Campylobacter jejuni, Clostridium perfringens, Bacillus cereus* and *Lactobacillus* spp. can be conducted.

6.2.2 Chemical analysis

Conclusion

In the chemical analysis, the results of this study provided information on the concentrations of BBP, DMP, DEHA, DnBP and DBP in food samples wrapped in plastic. More than 50% of the total amount of food samples analysed contained phthalates. The total amount of phthalate concentration ranged from 0.001 to 4.700 µg/kg and showed the migration level of phthalate esters from plastic wrappers into food. BBP was the most common plasticiser detected in all samples (ranging from 0.001 to 0.673 µg/kg), followed by DBP (ranging from 0.0418 to 0.611 µg/kg), DMP (ranging from 0.056 to 4.700 µg/kg), and DnBP (ranging from not detected to 0.925 µg/kg) and DEHA (not detected to 0.120 µg/kg). From the data of the results, it can be suggested that BBP and DBP have a more significant role in the food packaging industry in South Africa (Pretoria), than DMP, DnBP and DEHA. Overall, the highest concentrations of plasticisers were detected in cheese samples, which can be, attributed to the fat content of cheese. In general, the sources of these compounds in the food could be from printed ink, surfaces, gloves during food handling, plastic wrappers etc.

The following factors are concluded to have influenced the migration of plasticiser from plastic wrapper into the food: the fat form and content of the food, the temperature and duration of exposure of food to the film and the manner in which the food is wrapped. This includes food which is entirely over-wrapped in film, over wrapped on a polystyrene tray or a deep sided tray where the food is not intended to come into direct contact with the film.

Future research

Future research is recommended in the of study phthalates in food in South Africa with an increase in the quantity of samples tested, and adding different types of samples which are wrapped in plastic material. Duty *et al*. (2005) and van den Berg *et al*. (2003) indicated in their study respectively that "phthalates generally have low acute toxicity with short half lives of approximately 6~12 hours" and that they are "metabolized and eliminated within 48 hours of exposure to vertebrates and are, therefore, not strongly bioaccumulative." It is then recommended that future research be conducted to determine the health effects of consumers who regularly purchase plastic wrapped foods from the departmental stores found in the areas across South Africa.

Although this research topic focused only on phthalates in food wrappers as a specific chemical class, this should not be limited to only this group. Further research can focus on EDCs which occur cumulatively in the environment as complex mixtures, which must, therefore, be identified and included in the exposure risk assessments.

It is also necessary to determine how long it takes for the phthalates to migrate into the food from the time the food is wrapped with the plastic.

Recommendations

a) The use of alternative material to preserve and present food samples

The use of alternative packaging material can be one of the aspects to be explored. Many investigators such as Goulas *et al.* (2000) suggested the replacement of plasticised PVC in cheese packaging and home wrapping. Although, in the results obtained in this study, some of the levels of phthalates in the tested samples were insignificant in causing harm to the consumer; it is recommended that more efforts in substituting PVC films, which contain harmful phthalates, with PVC films, which exhibit a, lower potential for migration into foods. The use of secondary packaging, which prevents direct contact of the food sample with the plastic packaging, can also reduce the migration of phthalates in foodstuff.

It is also recommended that there should be a reduction of phthalates use in plastic packaging for food and replacement of plastic packaging with paper which will ultimately prevent the exposure of phthalate to human. The results of the study by Goulas *et al.* (2007) reported the implications for the food packaging industry:

(i) The surface treatment of plasticised PVC films photocross-linking, surface modification with nucleophilic substitution of chlorine by azide, plasma surface modification, etc.

(ii) Greater or complete replacement of common (monomeric) plasticisers with polymeric plasticisers.

(iii) Greater substitution of DEHA by ATBC in PVC cling films.

(iv) A trend towards using alternative flexible films with low-plasticizer content such as vinylidene chloride copolymerized up to 20% vinyl chloride (PVDC/PVC) (Saran) film containing only ATBC plasticizer (approximately 5% w/w).

According to Scottish Environment Protection Agency (SEPA, n.d.), there are steps which have been taken to restrict potential consequence of BBP exposure. This phthalate has been listed for risk assessment under EC law (793/93/EEC) also listed as priority sustenance for EC Water Framework Directive. This shows that more attention will be given to investigate the effect of this phthalate in food and its effects on human health.

However, more practical recommendations were stipulated by Goula *et al.* (2000) in a study on Kefalotyri cheese. It was proposed that people should get rid of about approximately 1mm thickness of cheese before consumption, to avoid cheese contaminated with plasticiser. The same recommendation holds for rindless cheeses such as Feta and for the retail pieces of cheese packaged in PVC cling film. This recommendation can also be adopted for the current study. Applicably, this would mean that, before consumption, the surface of the food

which is in contact with the plastic should be cut out to ensure low levels of phthalates which could have migrated into the food.

b) Food inspection

It is also recommended that health inspectors be appointed. They will monitor that small stores adhere to the microbial standards and storage standards stipulated by the Department of Health. This will also ensure that food be labelled correctly with expiry dates and that once the foods have expired, they can be discarded in order to protect consumers.

In the present study, one of the main objectives of the study was to investigate incidences and determine phthalate ester levels as well as microbial entities in food samples wrapped in plastic in Tshwane Metropolitan stores. The results obtained showed the presence of various phthalate esters in the three food sample types tested. According to literature available, phthalates have adverse health effects on animals (Gray *et al.,* 2000; Foster *et al.,* 2000; Gray *et al.,* 2001; Latini, 2005). These effects include carcinogenicity, foetal death, malformations, testicular injury, liver injury, anti-androgenic activity, teratogenicity, peroxisome proliferation and especially reproductive toxicity. However, little information is known on the effect in humans, although some studies lead to the change in regulations. It is recommended that limited number of phthalates be used in plastic materials. However, the results obtained do not generalise that all kinds of polony, cheese and vienna found in South Africa are contaminated.

Another objective was to compare phthalate levels with standards to determine compliance with US EPA and the South African Department of Health.

- It was found that the number of coliforms in vienna was 2.62 x 10^6 cfu/g and 2.04 x10^4 cfu/g for polony which exceeded the limit set by the Department of Health is not greater than 200 cfu/g.
- No *E. coli* were detected in any of the food samples; this was acceptable according to the standards set by the Department of Health of South Africa.
- High levels of viable bacteria were found in vienna samples. The total microbial count (TMA) ranged from 68000 to 103 000 000 cfu/g. According to the South

African Department of Health (n.d.), the proposed microbiological parameters to be used as guidelines for food set for processed meats (viennas, polony, etc) are stipulated as follows: the TMA should not be greater than 200 000 cfu/g. It can be seen that the microbial counts exceeded the stipulated acceptable limits.

- High levels of yeast were also found in polony samples; ranging between 3.7 x 10^4 to 3.5 x 10^6 cfu/g. The limit set by the Department of Health (provide reference!!!) is not greater than 1000/g which has also been exceeded.

Both hypotheses I and II where accepted as true according to the results obtained from the research work. It is however still recommended that further research be conducted in these areas of study in order to establish and compile more comprehensive data and also expand the research into other food samples that are wrapped or presented in plastic films that may contain phthalate esters.

The results give cause for concern and encourage the need for proper monitoring and inspection of the levels of organo-microbial entities in the South African food wrapped in plastic wrappers. This will promote good human health.

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APPENDIX

Figure 1 a - d below shows the trend of the presence of food spoilers in the four metropolitan regions of Pretoria. It can be seen that food spoilers were detected in different samples with variety in the amount detected in each site.

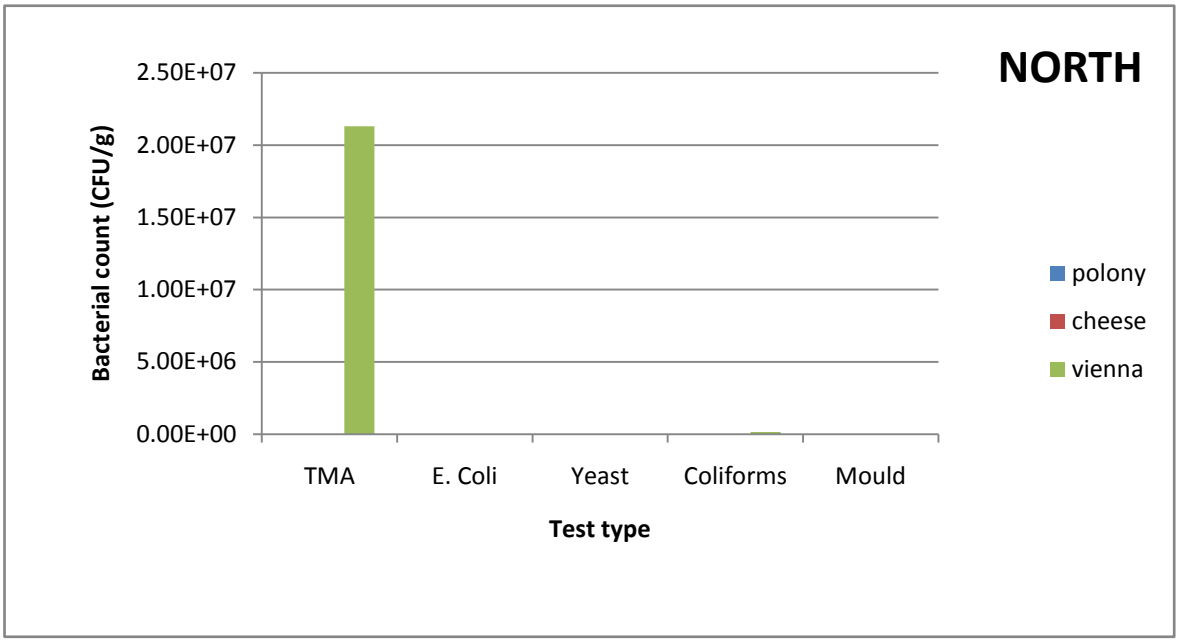


Figure 1a Pathogens and food spoilers in tested samples purchased from Pretoria North

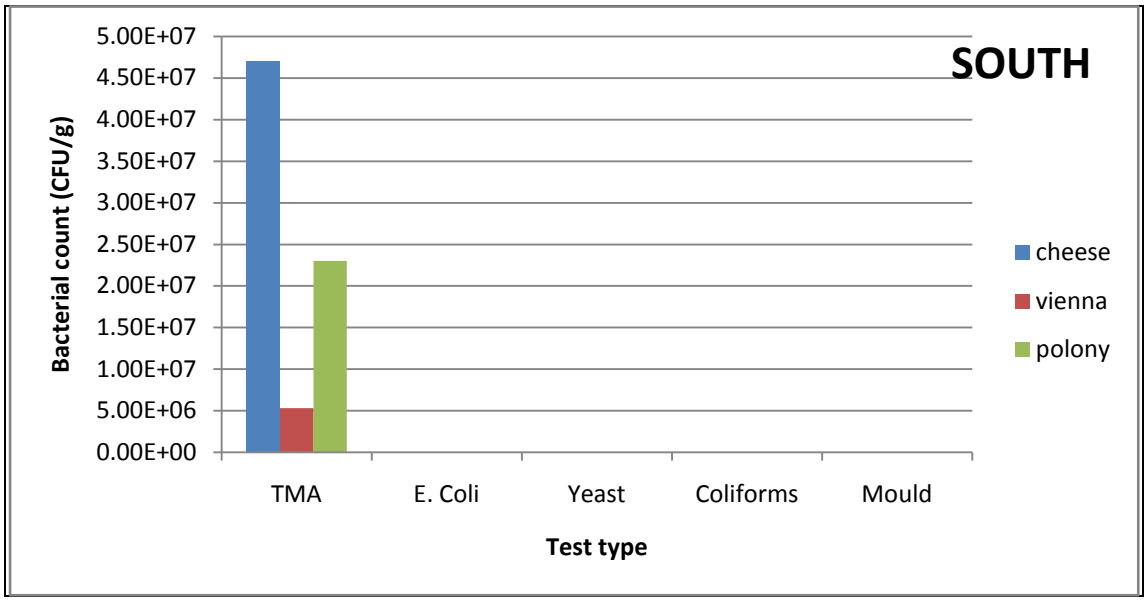


Figure 1b Pathogens and food spoilers in tested samples purchased from Pretoria South

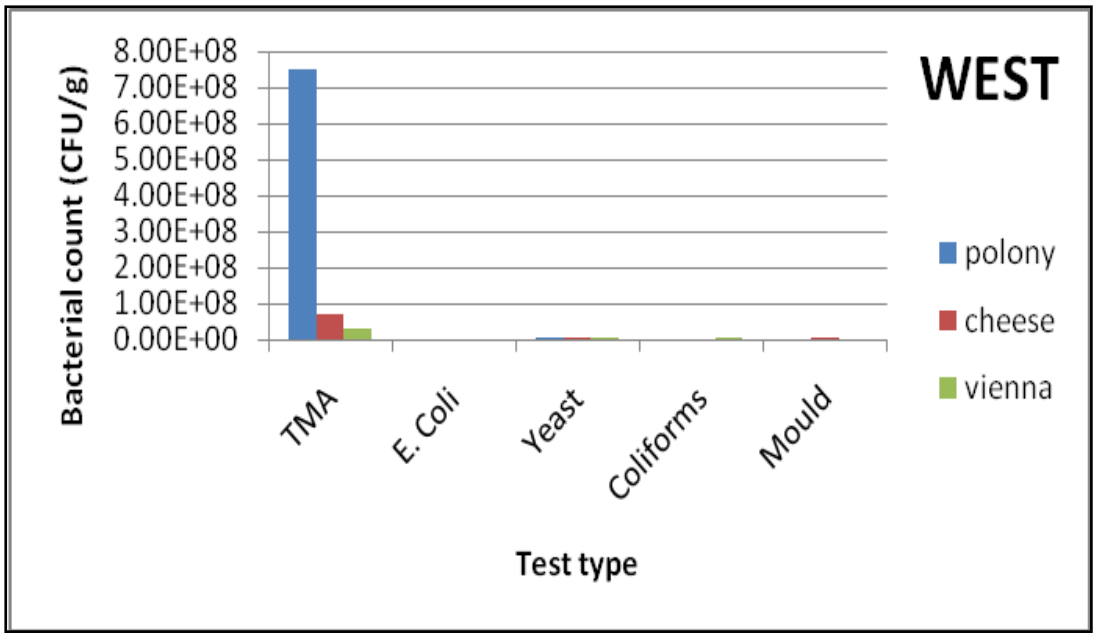


Figure 1c Pathogens and food spoilers in tested samples purchased from Pretoria West

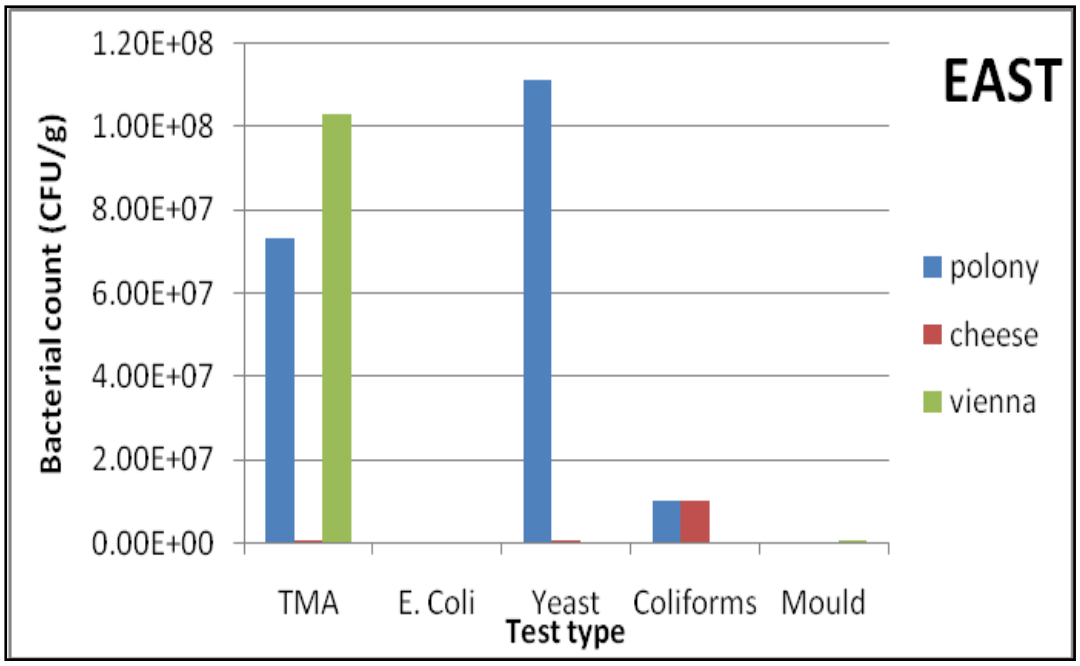
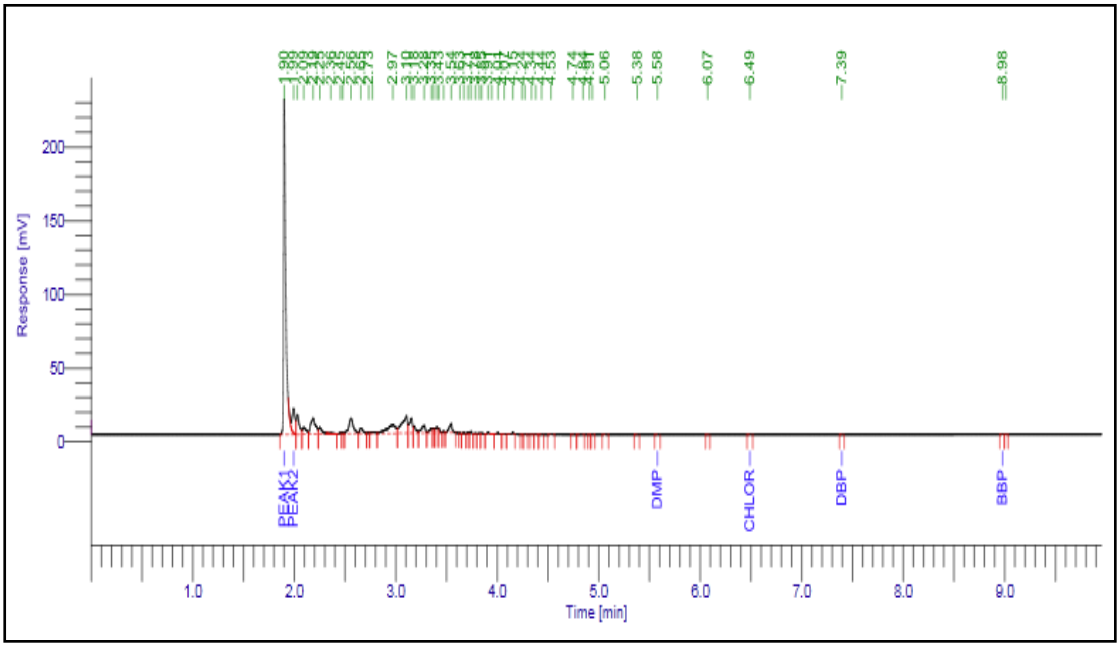


Figure 1d Pathogens and food spoilers in tested samples purchased from Pretoria East

Figure 2, 3, and 4 show the chromatogram with results from the three studied samples. It can be seen that there is an indication of the presence of phthalate esters at various retention times. More results are listed in Tables 4.6, 4.7 and 4.8.

(A)



(B)

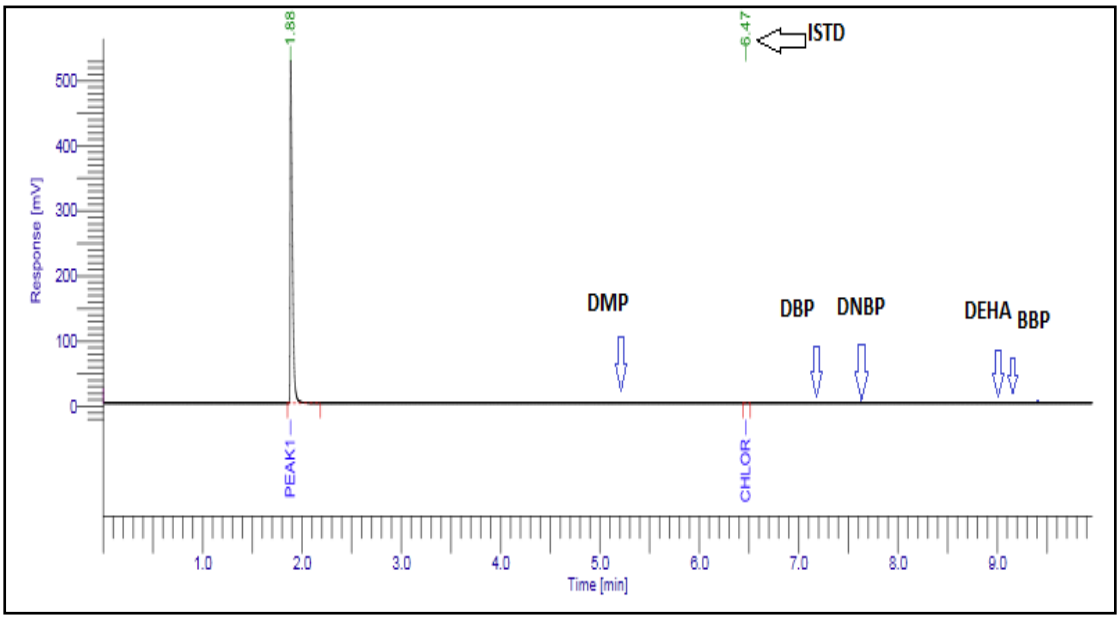
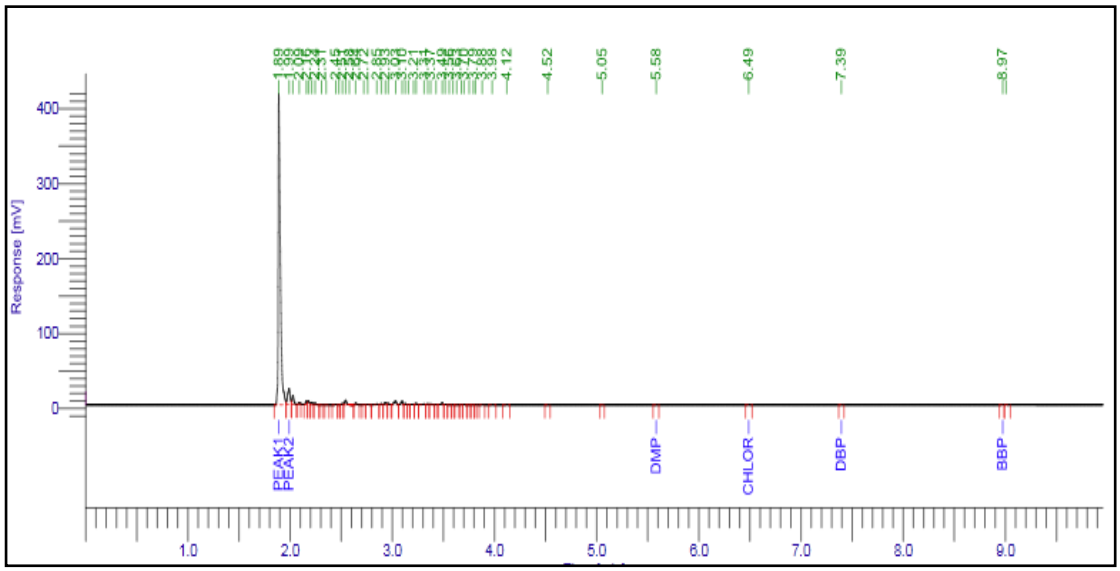


Figure 2 Chromatograms showing concentration of various phthalate esters in cheese sample from Pretoria East

(A) Sample of processed polony from Pretoria East showing phthalate concentration

(B) Blank sample (remote packaging contact) showing only internal standard and the absence of phthalates

(A)



(B)

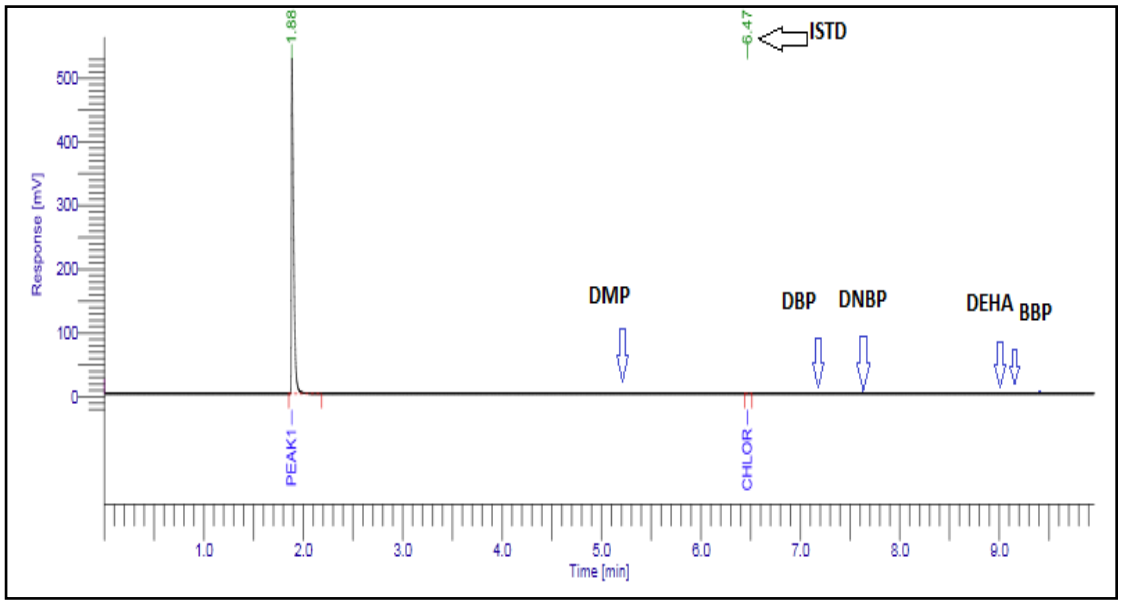
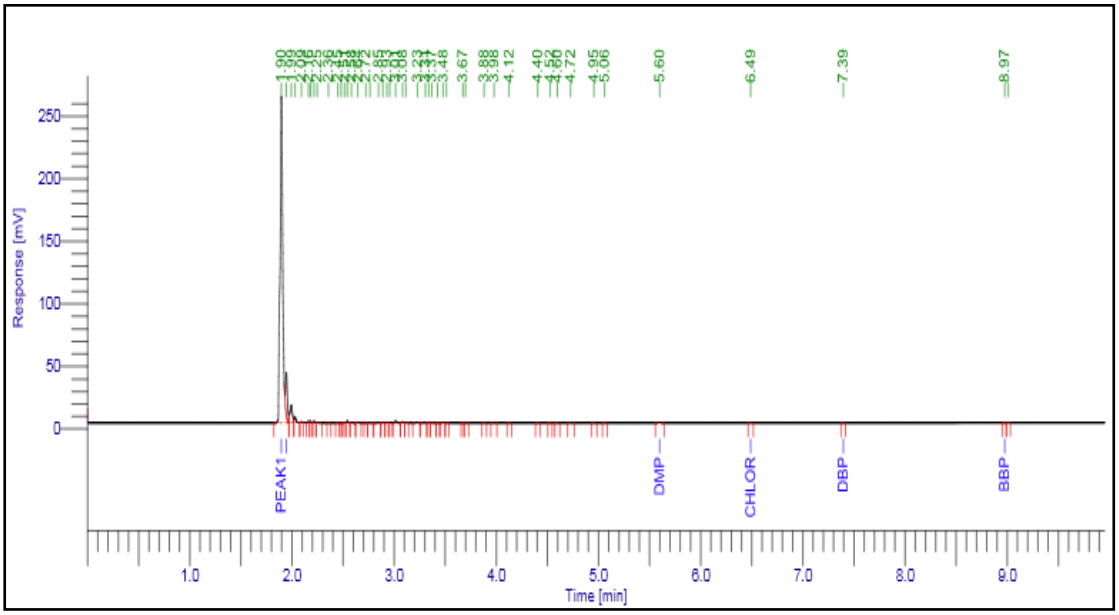


Figure 3 Chromatograms showing concentration of various phthalate esters in polony sample from Pretoria East

(A) Sample of processed polony from Pretoria East showing phthalate concentration

(B) Blank sample (remote packaging contact) showing only internal standard and the absence of phthalates

(A)



(B)

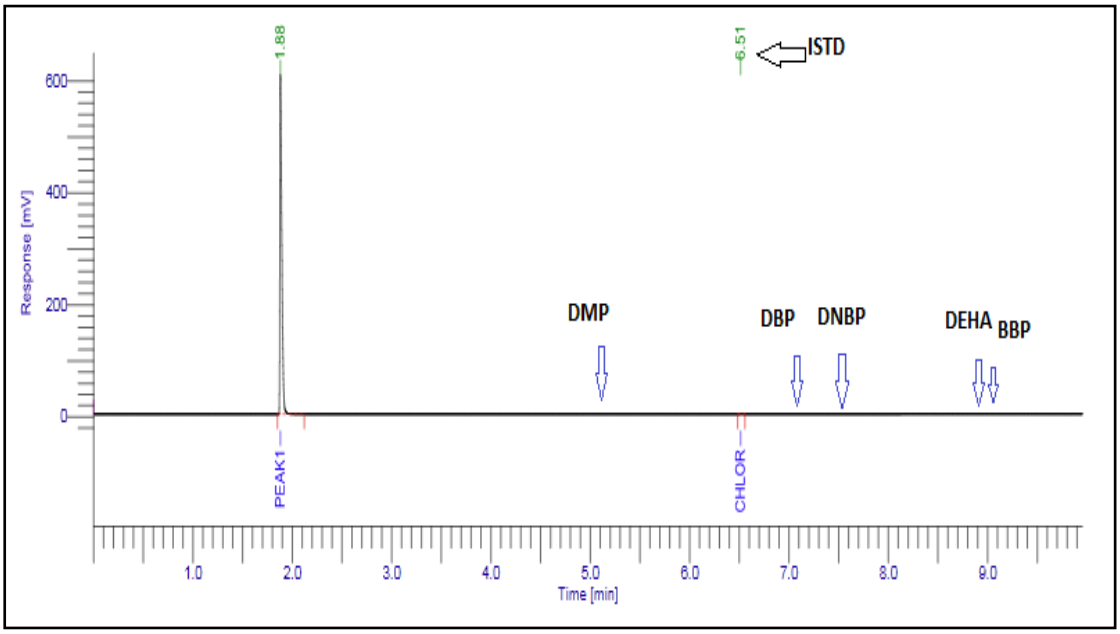


Figure 4 Chromatograms showing concentration of various phthalate esters in vienna sample from Pretoria North

(A) Sample of processed cheese from Pretoria North showing phthalate concentration

(B) Blank sample (remote packaging contact) showing only internal standard and the absence of phthalates

An example can be seen in Figure 5a and Figure 5b below which shows the chromatogram of all standards, and as compared to the chromatograms obtained from the test samples (see Figure 2, 3, and 4), it can be seen that there was a similarity in the elution times.

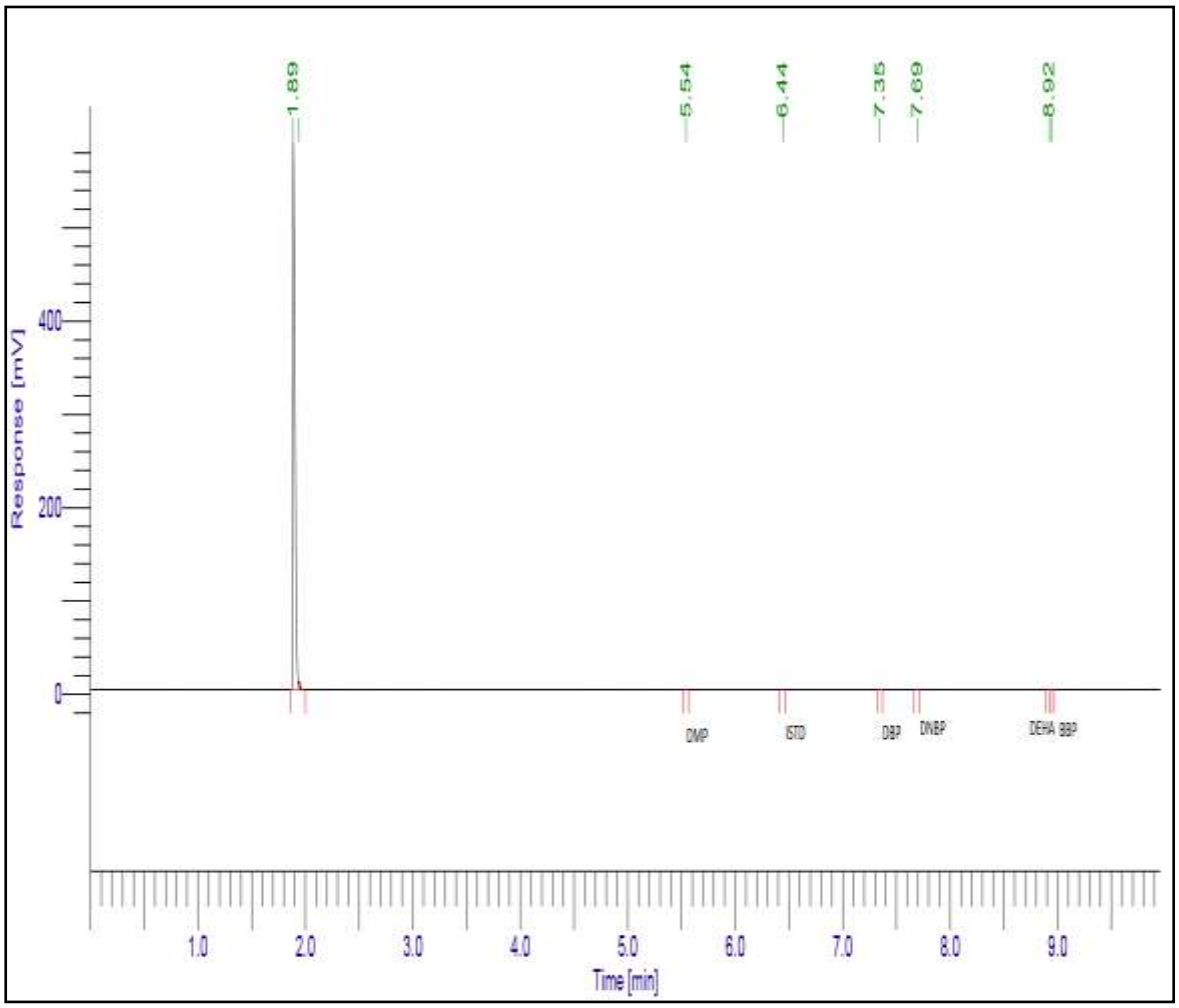


Figure 5a Chromatograms of Calibration Standard

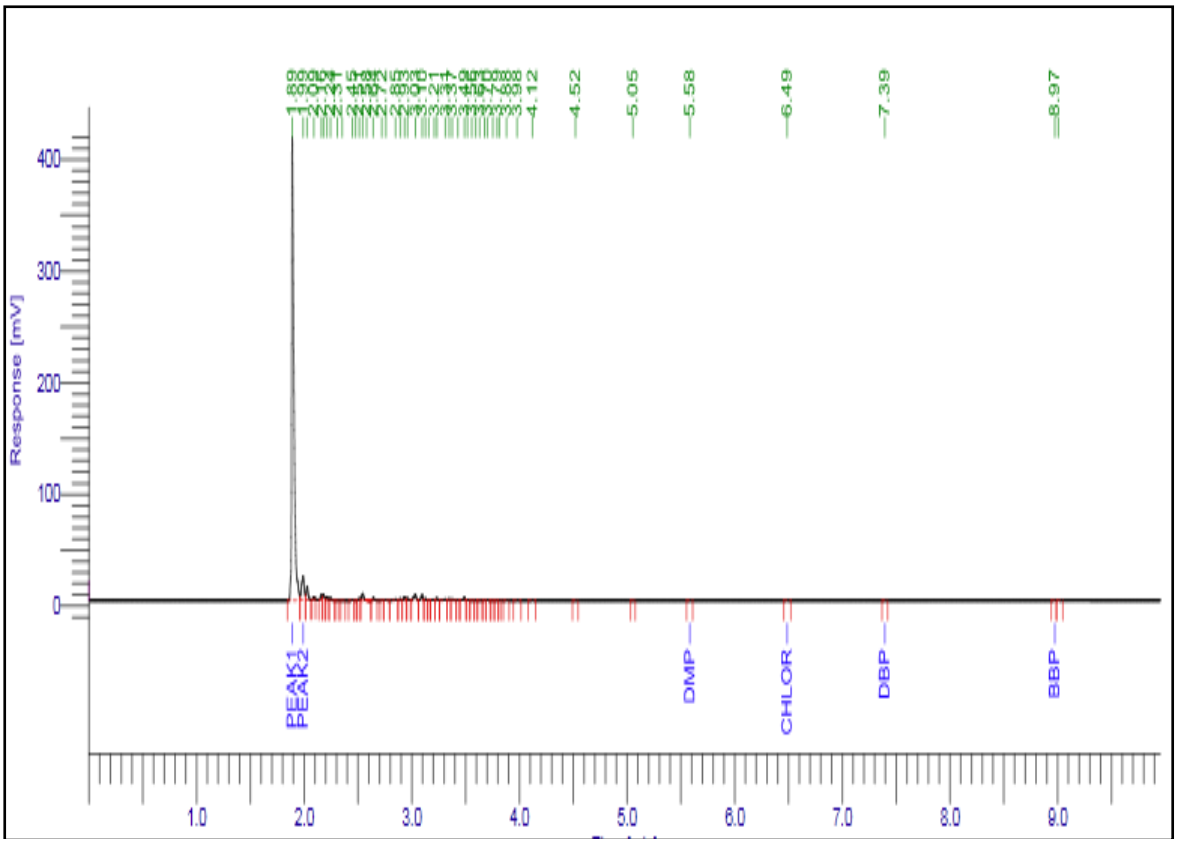


Figure 5b Chromatograms of contaminated polony extract.