Development of Natural Antioxidant Active Polymer Package from Extracts of *Monodora myristica* (Ehuru) for Lipid Food Preservation

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CERTIFICATION

This is to certify that this work 'Development of Natural Antioxidant Active Polymer Package from Extracts of *Monodora myristica* (Ehuru) for Lipid Food Preservation' was carried out by NWAKAUDU ADANZE ANGELA, (20114834568), in partial fulfilment for the award of degree of Ph.D in Food Processing, in the Department of Food Science and Technology, Federal University of Technology Owerri.

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DEDICATION

This piece of work is dedicated to Almighty God, who gave me the life, strength and all the resources to finish it.

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ABSTRACT

A natural antioxidatant active polymer package film was developed for lipid food preservation. Three active package films were produced, namely: film containing only ehuru antioxidant extract (EAE), α-tocopherol (AT) and a blend of EAE/AT using casting method. Different percentage compositions (0 - 5% w/w) of EAE were incorporated into polysulphone (PSF) resin, while 5% AT was incorporated into PSF resin which served as standard. A 5% equal combination of EAE/AT was also added to PSF to produce package film. Mechanical, thermal, morphology and barrier properties of developed films as well as the pure PSF film were determined and compared. The antioxidant ability of the developed films was investigated using 2,2- Diphenyl-1-picrylhydrazyl (DPPH) method. The ability of the films to stabilise lipid (peanut oil) against oxidation was tested by packaging peanut oil with the active films. The peroxide value (PV) and p-anisidine value) of the actively packaged and stored peanut oil were determined. Results of the barrier properties showed that the rate of permeation of gas in the EAE active films was shown to be reducing significantly (from 0.1256 to 0.0277)cm^3/s as concentration of EAE incoporated in the film increased, while films with blend of EAE/AT produced higher permeable films of (0.1079 cm^3/s). AT film was the most permeable (0.2209 cm^3/s). The spectrum of the FT-IR analysis confirmed the presence of esters, amides and amines in the absorption peaks of 3526, 3302.4 and 3697.5 cm^{-1}. Absorption peak of 1736.9 cm^{-1} for the ester and 1684.8 cm^{-1} for amide. The SEM microstructure revealed that the porous film of the pure PSF reduced as concentration of the EAE added increased from 0% - 5%. The incorporation of 5% EAE , produced very smooth and homogenous surface film without apparent phase separation. The melting point of EAE active film was established at 210 ^oC compared to 230 ^oC for the pure PSF. The AT active firm recorded a higher melting temperature of 247 ^oC. The oxidation results showed a significantly ($p < 0.05$) lower rate increase in oil samples packaged with the developed films than the control sample. Results also revealed that at the end of nine weeks of storage, peanut oil packaged with active film containing only EAE natural antioxidant showed the lowest lipid oxidation by recording the lowest peroxide and p-anisidine values of 9.82±1.50 meq/kg and 30.45±0.00 respectively. The effect of concentration of the EAE on the oxidation of the peanut oil showed that their is no signifcant difference ($p > 0.05$) in the 2.5% and 5% EAE on the PV and P- anisidine values of the packaged oil sample, hence concentration between 2.5 and 5% could be used for the active film. Considering the mechanical properties of the active package, 5% EAE produced the most flexible film with percentage elongation at break (EAB) of 3.221% and best antioxidant activity of 48.097%. The overall results showed that the developed Ehuru antioxidative package had demonstrated the potential of slowing down the lipid oxidation and therefore could be used to preserve lipid foods like peanut oil.

Key words: Active Polymer package, Natural Antioxidatant, *M*onodora *m*yristica, Spice extract, Lipid preservation

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Food losses due to oxidation constitute a great economic challenge for the food industry and consumers alike. Food spoilage as a result of oxidation is characterised by alterations of nutritional and sensory characteristics of food such as production of off-flavours and off-odours. It also involves undesirable changes in texture, colour and chemical changes (production of radicals) that can harm human. As a result, new packaging technologies have been studied in order to provide good quality, safer food products with longer shelf life (Otoni, Espitia, Avena-Bustillos, & McHugh, 2016). Among these technologies is Modified Atmosphere Packaging (MAP). This system of packaging involves changing the gaseous atmosphere surrounding a food product and employing packaging materials with appropriate level of gas barrier to maintain the changed atmosphere. It is a post-harvest technique used to increase the shelf life of fresh produce (Joshi, Warbi, Valverde, Tiwari, & Cullen, 2018). Another new packaging system is active packaging, where the packaging material interacts with the packaged food in a desirable way, overcoming the passive role of just containment and protecting food products from the outside environment (Ahmed *et al*., 2017)

Active packaging with antioxidant properties have taken the centre focus of food packaging technology research because of the fact that oxidation and microbial contamination are the most significant problems affecting food quality and safety. Active packaging systems are special food polymer packaging materials that contain significant amounts of (active) components that provide desirable preservative roles to food products. The process is termed "active packaging"

because the package act as an antioxidant amongst its other functions. (Arrieta, Lopez, Hernandez, & Rayon, 2014; Shojaee-Aliabadi *et al*., 2013)

This new trend came into place because of several reports that the direct addition of natural antioxidants into the bulk food do act as pro-oxidant while chemical preservatives have been implicated to be detrimental to human health. These adverse effectsrange from the carcinogenic effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) to interference of BHA in the human hormone (endocrine) system (Samsudin *et al*., 2017; Tátraaljai, Kirschweng, Kovács, Földes, & Pukánszky, 2013)

Alot of studies have been conducted on the utilization ofplant polyphenols as alternatives to chemical antioxidant agents (BHT and BHA).These chemical antioxidant agents are reported to be susceptible to migration during heating or long term storage because they are not covalently bound to the polymer. Therefore, there is this growing interest in the scientific community in finding and replacing chemical antioxidants with natural ones as preservatives in food and food contact materials, These natural antioxidants are considered safe since they belong to the group of foods generally regarded as safe (GRAS) (Doshi, Adsule, & Banerjee, 2015).

Lipid oxidation is considered a principal means of deterioration or spoilage of oily foodstuffs such as vegetable oils, animal fats, flavourings, nuts, processed meats and snack products, etc. The need for antioxidants is not only limited to high-lipid foods but also includes products such as cereals which contain only 2-5% lipid components. Oxidation not only negatively influences the chemical, sensory (flavor, texture and color), and nutritional properties of edible oils and fatty foods, but also produces free radicals and reactive oxygen species (ROS). These free radicals and ROS have been reported to be associated with most health

problems encountered in man like carcinogenesis, inflammation, aging, cardiovascular disorders, motility, chemotherapy response, and drug resistance (Huang & Freter, 2015). Oxidation therefore, plays a very important role in determining what a particular oil or fat can be used for as well as its shelf life (Anwar, Chatta, & Hussain, 2007; Das Sarma, Mallick, & Ghosh, 2010; Tian, Decker, & Goddard, 2013).

Hydroperoxides, which are the first products of autoxidation, are colourless, tasteless, and odourless. These products break down to low-molecular-weight compounds that are characterized with rancid flavours and odours. A lot of food additives in combination with food packaging strategies are presently employed to prevent these harmful oxidative reactions within food systems.

Synthetic (BHA, BHT) and natural antioxidants (ascorbic acid, tocopherol) are added directly to foods as primary antioxidants, which donate hydrogen atoms to quench peroxide radicals before they can further react with unsaturated lipids. Phenolic antioxidants are stable due to the fact that they form a radical with very low reactivity, due to delocalization of their unpaired electron on the aromatic ring. With this, they exhibit no further potential to react with lipids after hydrogen abstraction unlike their oxidation radicals. Even though lipid foods have some quantity of inherent defence system (antioxidant) that resists oxidative damage due to ROS, supplementing this natural defence mechanism with external antioxidants offers a better protection against the risk of certain oxidative deterioration.

It has been established that there is great antioxidant potential and health benefits contained in natural products like green tea, rosemary, oregano,spices like ehuru, herbs, clove, blueberries, barley husk, and other plants (Colon & Nerin, 2012; Pereira De Abreu, Losada, Maroto, & Cruz, 2010; Tátraaljai *et al*., 2013)

Several works have been reported on the incorporation of tocopherol (Vitamin E) and ascorbic acid (Vitamin C) as active agents into a polymer (Gemili, Yemenicioğlu, & Altınkaya, 2010; Noronha, De Carvalho, Lino, & Barreto, 2014; Siró *et al*., 2007). Other works on natural antioxidants from leafy spice extracts such as green tea extracts *(Camellia sineensis L.)* have been reported by Colon & Nerin (2012) and López-de-Dicastillo *et al*., (2011), while works on rosemary (*Rosmarinus officinalis L*.), have been reported by Sánchez-Escalante, *et al*., (2001); extracts of oregano also known as *"thyme"* (*Origanum vulgare L.)* was reported by (Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011) mint *(Mentha spicata L.)* and extracts of sage (*Salvia officinalis L*.) as active agents incorporated into polymer food packaging for preservation have all been reported. Other works dwelt on the incorporation of root spices such as Cucumen (*turmeric*) and Ginger *(Zingiber officinale Rosc.),* into polymer food packaging materials. (Gemili *et al*., 2010). However, till date, no work on the use of seed spices has been reported as active agent incorporated in polymer film packaging material. Interestingly, Ehuru (*Monodora myristica*) extracts has been reported by researchers to exhibit a potent antioxidant activity and also effectiveness for achieving high sensory scores and lowering lipid oxidation (Akinwunmi & Oyedapo, 2013; George & Osioma, 2011; Okonkwo & Ogu, 2014). Therefore, there is need to investigate the performance of this indegenous seed spice (Ehuru) as active additive into polymer packaging material.

1.2 Problem Statement

Most of the food being packaged with polymeric materials are faced with oxygen and UV light permeation which leads to free radical chain formation, with consequent food deterioration. Chemical/synthetic antioxidants**;** butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) used in preserving foods have been implicated in carcinogenicity, and hence a safety challenge to consumers.

In most foods, the surface growth of peroxides as a result of oxidation is the major cause of lipid food spoilage. Since oxidation is a chain reaction process, an extremely proactive approach must be taken to prevent or limit the initiation step. This implies that methods that can eliminate surface peroxides as soon as they are formed would greatly slow downthe process of oxidation and ensure shelf-life extension. This approach would eliminate the need for further addition of antioxidants in the bulk of the food. This research is aimed at producing an antioxidative active package that would adress this probem

1.3 Objectives of Study

The main objective of this research is to develop a natural antioxidant active polymer package from extracts of *Monodora myristica* (ehuru) for Lipid food preservation.

The specific objectives are as follows:

i. To determine the phenolic compositions of the spice
ii. To prepare the ehuru extract from the seed spice
iii. To develop natural antioxidant active Polysolfone (PSF) packaging films.
iv. To determine the antioxidative capacity of the developed active packaging films
v. To establish the effect of the natural antioxidant additive on the mechanical, thermal and permeability properties of the polymer food packaging materials
vi. To assess the effectiveness of the active package in reducing lipid oxidation in food

1.4 Justification of study

Having observed the negative effects of oxidation, leaching and safety of the conventional antioxidants in food packaged with polymer materials, there is need to produce novel polymeric packaging material. These materials containing natural antioxidative fillers may be safe, help to reduce food wastage, food

poisoning and allegic reactions because no chemical additives or preservatives are added into the food. The natural antioxidants when released into food, increase the content of bioactive compounds in the food products instead of constituting a toxicological risk as occurs with synthetic antioxidants. The use of natural antioxidants from Ehuru will add industrial value to these indegenous spice. It will also promote shelf stability of packaged oily foods especially in the rural areas that lack electricity.

1.5 Scope of study

The scope of this project covers the production and characterisation of polymer PSF active film using ehuru as the source of natural antioxidant. Also covered is the storage study on the actively packaged fresh peanut edible oil packaged with the developed active polymer film, in order to monitor the oxidation rates of the packaged oil. The effect of concentrations of the natural antioxidants on the mechanical, thermal and permeability properties was also covered in the work.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to Food Packaging

Food Packaging is defined as enclosing food to protect it from physical, chemical and biological spoilage, to provide consumers with ingredient and nutritional and safety information and also the preparation of foods for shipment and marketing (Robertson, 2012). The goal of food packaging is to contain food in a cost-effective manner that satisfies the needs of the industry and also meets with the desires of the consumers, maintains food safety, and minimizes environmental impact (Marsh & Bugusu, 2007). The successful employment of food packaging can uniquely help to better product safety and quality, this makes this area an important concern to the food processing industry.

2.1.1 Functions of Food Packaging Materials

Food packaging performs a variety of tasks at the same time satisfies many demands and requirements. Basic packaging reqirements include good marketing properties, reasonable price, technical feasibility, suitability for automatic packaging machines, seal-ability, suitability for low contact, low environmental stress, recyclability and refilling (Ahvenainen, 2003). A packaging material must satisfy each of these requirements both effectively and economically. Furthermore packaging has a more significant role in the preservation of and ensuring the safety of the food.This is observed in active packaging.

Packaging maintains the benefits of food processing after the process is complete, enabling foods to travel safely for long distances from their point of origin and still be physically intact and wholesome at the time of consumption. The primary purpose of food packaging is to protect the food against attack from oxygen, water vapor, ultraviolet light, and both chemical and microbiological contamination. Therefore, food packaging contributes to extending of the shelf-life and

maintaining of the safety of the food product. Four primary functions of packaging have been identified as containment, protection, convenience and communication.

(a) Containment

The containment function of packaging makes a huge contribution to protecting the environment from the myriad products which are moved from one place to another on numerous occasions each day in any modern society. Faulty (or under-packaging) could result in major pollution of the environment. Any assessment of food packaging impacts on the environment must consider the positive benefit of reduced food wastes throughout the supply chain. Kenneth & Betty (2007), noted that significant food wastage has been reported in many countries ranging from 25% for food grains and 50% for fruits and vegetables.

(b) Protection

This is often regarded as the primary function of the package to protect its content from outside influence and damage such as water, moisture, vapour, gases, odours, microorganisms, dusts, shocks, vibration and compressive forces. Kennetth & Betty (2007) also noted that packaging must protect the food from biological agents such as rats, insects, and microbes; from mechanical damage such as product abrasion, compressive forces, and vibration; and from chemical degradation such as oxidation, moisture transfer, and ultraviolet light. Food packaging can retard product deterioration, retain the beneficial effects of processing, extend shelf life, and maintain or increase the quality and safety of food.

(c) Convenience

Convenience features such as ease of access, handling, and disposal product, visibility and resealabilty, greatly influence package innovation. As a convenience, packaging minimizes the effort necessary to prepare and serve

foods. New closure designs supply ease of opening, sealability, and special dispensing features.

In the bid to protect the food by putting in a container and also communicating a message to the consumer, it is observed that the food quality and safety can be jeopardized or affected because the packaged material selected has an effect or interact with the food, hence some of the criteria for package selection becomes important.

(d) Communication

A package is the face of a product and often is the only product exposure the consumers experience prior to purchase. Consequently distinctive and innovative packaging can boost sales in a competitive environment. Packaged food must be identified for consumer use mainly with label text and graphics. It can also be shown with the food package's shape such as the coca-cola bottle or the can of spam. Other well known food package shapes include the potato chips bags and milk bottles. These packages also gives details of nutritional information, (if they are approved by agencies like NAFDAC), manufacturer, product size (volume or weight-based), and the universal product (UPC)(Yam, 2009).

(e) Environmental friendliness and disposability

Therefore, according to these conditions, packaging design and development includes not only the industrial design fields, creating and marketing tools, but also the areas of engineering and environmental science. Preservation, convenience and other basic functions of packaging are important but its disposal should also be treated as an important aspect of packaging development. This is a problem that can confront us in the future (Stillwell, Canty, Copt, Mintrone, & Arthur, 1991)

Criteria for Selection of Food Packaging Materials

Robertson (2012), listed a number of criteria that should be considered when selecting a packaging system for a food. These include:

i. The stability of the food with respect to the deteriorative chemical, biochemical and microbiological reactions. The rates of these reactions depend on both intrinsic (compositional) and extrinsic (environmental) factors.
ii. Compatibility of the package with the selected method of preservation.
iii. The environmental conditions to which the food will be exposed during distribution and storage. The ambient temperature and humidity are the two most important environmental factors and they dictate the barrier properties required of the package.
iv. The nature and compositions of the specific packaging material and its potential effects on the intrinsic quality and safety of the packaged food as a consequence of the migration of components from the packaging material into the food.

2.3 Types of Packaging and Packaging Materials

Packaging can be classified into primary, secondary and tertiary packaging. Primary packaging is the main package that holds the food that is being processed. Secondary packaging combines the primary packages into one box being made. Tertiary packaging combines all of the secondary packages into one pallet as shown in Table 2.1. Packaging materials comes in the form of Metal, glass, paper, polymers: Plastic (polyethylene, Polypropylene, polystyrene, polysulphone, Polyethylene terephthalate (PET)and biopolymers (cellulose,poly amide, polactic acid (PLA), bio- Pet. Plastic is highly prefered to other packaging materials because of its lightness, flexibility, durability and low cost, according to world data on plastics, 35 -40% of 300 million tons of plastic produced every year are used for packaging alone (Yam, 2009).

2.4 Food Grade Polymeric Packaging Materials

Materials traditionally used in food packaging include leaves, plastics, glass, metals (aluminum foils and laminates, tin plates and tin-free steel), paper and paper board, however, multiple types of plastics are beingused as materials for packaging foods, including polyolefin, polyethylene, polyester, polypropylene, polyvinylchloride, polyvinylidene chloride, polystyrene, polysulphone, polyamide, and ethylene vinyl alcohol. Lau & Wong (2000), noted that even though there are more than 30 types of plastics used as packaging materials, polyolefins and polyesters are the most common.

Table 2.1: Types of packaging

Packaging type	**Type of container**	**Food examples**
Aseptic packages	Primary	Liquid whole eggs
Bags	Primary	Potato chips
Boxes	Secondary	Box of Coca-Cola
Cans	Primary	Can of Tomato soup
Cartons	Primary	Carton of eggs
Flexible packaging	Primary	Bagged salad
	Tertiary	A series of boxes on a single pallet used to transport from the manufacturing company to the distribution Centre.
Wrappers	Tertiary	Used to wrap the boxes on the pallet for transportation

Source:(Marsh & Bugusu, 2007)

(a)Polyolefins

Polyolefin is a collective term for polyethylene and polypropylene, the two most widely used plastics in food packaging. They both possess a successful combination of properties, including flexibility, strength, lightness, stability, moisture and chemical resistance and easy processabilty, and are well suited for recycling and reuse (Yam, 2009).

(b) Polyethylene (PE)

The simplest and most inexpensive plastic materials made by addition of polymerization of ethylene is polyethylene. There are two basic categories of polyethylene; high density polyethylene (HDPE) and low density polyethylene (LDPE)(Balakrishnan, Thomas, Pothen, Thomas, & Sreekala, 2015).

HDPE is stiff, strong, tough, resistant to chemicals and moisture, permeable to gas, easy to process and easy to form. Uses of HDPE includes bottles for milk, juice and water; cereal box liners; margarine tubs, trash and retail bags. Linear low density polyethylene (LLDPE) is flexible, strong, tough, easy to seal and resistant to moisture (Simpson & Vaughan, 2001). As a result of LDPE's relative transparency, it is predominantly used in film applications and in applications where heat sealing is necessary. Bread and frozen food bags, flexible lids, and squeezable food bottles are examples where LDPE films are used. PE are sometimes reused both for grocery and non grocery retail. HDPE containers, especially milk bottles, are the most recycled among plastic packages.

(c) Polypropylene (PP)

Polypropylene (PP) is another addition polymer which in its bulk state is harder, denser and more transparent than PE. It has good resistance to chemicals and a good barrier for water vapour. It has a high melting point of 160^{o}C which makes it suitable for applications where thermal resistance is required, such as hot-filled and microwavable packaging. Popular uses include yogurt containers and

margarine tubs. When used in combination with oxygen barrier such as ethylene vinyl alcohol or polyvinylidene chloride, PP provides the strength and moisture barrier for catsup and salad dressing bottles (Dadbin, Noferesti, & Frounchi, 2008).

Polysulfone: Structure and Properties

Polysulfones are a class of engineering polymers with high thermal, oxidative and hydrolytic stabilitiy. They are amorphous, transparent thermoplastics that can be molded, extruded, or thermoformed into a wide variety of shapes.The high thermal stability is provided by the diphenylene sulfone group. It imparts high strength, high resistance to oxidation, and excellent flame retardancy but makes the polymer rigid. Flexibility in the backbone of the polymer is provided by ether linkages. These ether linkages also add to the thermal stability. Many commercial grades can tolerate high temperatures for a long period of time. Some grades, like polyphenylsulfone, are extremely tough and have very high impact strength, comparable to polycarbonate.

Polysulfones are highly resistant to aqueous mineral acids, bases, and oxidizing agents and are fairly resistant to many non-polar solvents. However, polysulfones are not resistant to low polar solvents, such as esters, ketones, aromatic and chlorinated hydrocarbons.

The usual grades have a good melt stability which permits fabrication by conventional thermoplastic processing methods. Figure 2.1 shows the structure of polysulfone.

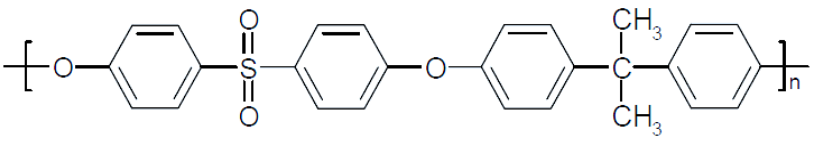


Fig 2; Structure of Polysulfone

2.4.3 Properties of polysulfones

Polysulfones have good resistance to aqeous mineral acids, alkali and salt solutions, oils and greases. Their high biocompatibility and ability to be sterilized makes them highly suitable for medical applications. Most grades can withstand long term exposure to hot chlorinated water. Several grades have received approval for food contact and drinking water.

Due to their superior resistance to chemicals and high temperatures, polysulfones are an excellent choice for components that are exposed to high temperatures and corrosive media. Examples include, internal components of coffee machines and battery containers.

Some (modified) polysulfones have a high permeability and permselectivity and are used as membrane materials for gas separation.

2.5 Barrier Packaging Materials

Packaging materials like glass and metal are considered excellent barriers to oxygen. Polymer packaging also can be used to reduce permeation of oxygen. High barrier films, such as ethylene vinyl alcohol, aluminium metallization and, most recently, clay Nano composites have been used to reduce oxygen permeability (Iheaturu & Ofoegbu, 2018). Similar to aluminium metallization, deposition of a thin layer of low permeability silicon oxide on a thermoplastic substrate has been used to greatly improve barrier properties. The oxygen permeability of these laminated films is very low relative to most polymer films, but still several orders of magnitude higher than that of silica glass. The presence of oxygen can be limited by vacuum or modified atmosphere packaging combined with high-barrier packaging materials, although it can seldom be completely eliminated because oxygen dissolved in the food at the time of packaging cannot be evacuated. Iron-based oxygen scavenging sachets are used

for their ability to preferentially oxidize, resulting in removal of oxygen from the package.

Products that are susceptible to oxidation, such as beer and wine, have traditionally been packaged in pigmented glass bottles. Pigmented high density polyethylene (HDPE) has been used in milk packaging to limit the degree of product photo oxidation. UV light absorbers have been added to polymer packaging of fruit beverages to allow delivery of 100% of the recommended daily allowance of vitamin C without the ascorbic acid experiencing the typical oxidation to dehydroascorbic acid.

Reterding the process of lipid oxidation process with antioxidant agentsis of great importance in protecting the quality of food products that contain unsaturated fatty acids, from possible deterioration. Over the past years, the food industry has used synthetic antioxidants, such as Butylated hydroxyanisole, butylated hydroxytoluene, propylgallate, tert-butyl hydroquinone and ascorbyl palmitate. Due to the presumed casinogenic potentials, consumers synthetic antioxidants food manufacturers now try to avoid or reduce to the bearest minimum their use as additives. The challenge has generated need to find out safer alternative sources of antioxidants for foods.

various packaging techniques have been introduced into the food industry for protective functions. They include the modified atmospheric packaging (MAP) and Active packaging systems. MAP had a challenge in preservation of lipid products because of the presence of residual oxygen with the ability to initiate deterioration. The recent packaging system in active packaging was invented to correct the flaw in MAP.The most appriopriate method of removing oxygen from a food package depends on the nature of the food, the packaging machinery, and the method of distribution.

2.6 Active Packaging

Active packaging as defined by Ganiari, Choulitoudi, & Oreopoulou (2017) is 'packaging in which subsidiary constituents have been deliberately included in or on the packaging material or the package headspace to enhance the performance of the package system' This emphasizes the importance of deliberately including a substance with the intention of enhancing the package. It is a system in which the product, the package, and the environment interact in a positive way to extend shelf-life. Unlike traditional packaging, which must be totally inert, active packaging is designed to interact with the contents and/or the surrounding environment in order to perform or provide some desired role other than only barrier to outside influences. For food contact materials to be active, they are designed by deliberately incorporating "active" components that would release or absorb substances intended to be released into the food or to absorb substances into or from the packaged food or the environment surrounding the food. So many works have been done illustrating this definition (Sobral & Luis, 2017; Arrieta, López, Ferrándiz, & Peltzer, 2013). According to Yam, Takhistov, & Miltz (2005), reported that a package is termed active when it performs some desired roles in food. It is successfully used to increase beyond the conventional package, the shelf-life of processed foods and meet consumer demands in terms of providing high-quality products that are also fresh and safe (Prasad & Kochhar, 2014).

Active packaging is an extension of the protection function of a packaging material specially against oxygen and moisture (Pereira De Abreu *et al*., 2010). During the past decade, active packaging has experienced significant growth and this change as innovation has challenged the normal traditional food and beverage packaging. Food polymer packaging can serve as a reservoir or matrix from which active ingredients are delivered in a controlled manner into the product. Han, (2013) categorized active packaging into adsorbing and

releasing systems, Antimicrobial preservative releasers, antioxidant releasers, and flavouring and aroma emitters. These are examples of active packaging systems for preservation, shelf life and quality extension of foods. Pereira De Abreu *et al*., (2010) highlighted on the importance of active packaging films containing natutral antioxidants. Several reviews regarding this subject can be found in the literature.

2.6.1 Active Packaging using Sachets and Pads

An older method of absorbing emitted gases from a package headspace, has commonly been through the use of sachets and pads. Sachets were developed in the 1970's in Japan. For oxygen scavenging, the sachets essentially utilize the process of rusting, or the oxidation of iron compounds in the presence of oxygen and water. Iron based scavengers typically do not pass the metal dictector inspections on most packaging lines, and in these incidences ascorbic acid is advantageous. Oxygen absorbers in sachets are commonly found in meat and poultry products, coffee, pizzas, baked goods and dried foods. Sachets that absorb carbon dioxide along with oxygen are also available and are most commonly found in roasted ground coffee packages (Fernández, Picouet, & Lloret, 2010). Some sachets are capable of emmiting ethanol as an antimicrobial agent to extend shelf life of high moisture bakery products. Drip absorbent pads may be used in packages containing meats that are likely to leak after the temperature fluctuations. These pads prevent the growth of moulds or bacteria by absorbing water into super-absobent polymer granules placed between two layers of microporous non-woven polymer (Hogan & Kerry, 2008). Athough sachets work well in many applications, they are not appropriate for every situation. Sachets cannot be used in liquid foods. They may not be used in a package made of flexible film, as the film would cling to the sachet and prevent it from performing its function. Sachets have the risk of accidental ingestion by consumers and this may account for their limited commercial success.

2.6.2 Active Packaging Materials Containing Active Components

Ozdemir & Floros (2004), have highligted recent attempts at active scavenging and have focused on incorporating the scavenger into the packaging material itself. This methods has potential for use in polyethylene terephthalate (PET) bottles and can be included in many plastic containers and closures. Adding scavengers to the plastic rather than a sachet can save many problems. For example, in a packaging film that is tight fiting such as a cheese pack, a sachet to absorb oxygen cannot be used because the tight fitting plastic would stiffle its functionality. Incorporating oxygen absorbing materials into the plastic components of the packaging material could be more efficient. One way in which oxygen absorbers are being incorporated into plastic materials is the use of a polymer based absorber that is coextruded in various packaging structures. The oxygen absorber is activated via UV light so that the scavenging capacity is not exhausted before the product shelf- life (Huff, 2015). Some systems developed so far use iron -based chemistry in their packaging material. Flavor enhancers are also being used in active packaging. It has been known for decades that packaging materials can scalp or absorb flavors from foods such as fruit juices. scalping is now being used in a positive way to absorb unwanted flavors and odors (Vilela, Silva, Medeiros, Fátima, & Soares 2013).

2.6.3 Active Packaging Materials Containing Enzymes

Approaches to oxygen scavenging include the addition of enzymes to the surface of films or bottles. Specifically, glucose oxidase and catalase can be added. When water is present, the glucose oxidase will oxidize glucose to gluconic acid and hydrogen peroxide. Then catalase will convert the hydrogen peroxide to water and oxygen. The net result is a reduction in oxygen. These enzymes can be added to the surface of polyethylene or polypropylene. The basic reaction for enzymes to reduce oxygen levels are shown in equations 2.1 and 2.2

$$2G + 2O_2 + 2H_2O \longrightarrow 2GO + 2H_2O_2 \quad (2.1)$$

$$2H_2O_2 + \text{Catalase} \longrightarrow 2H_2O + O_2 \quad (2.2)$$

where, G is the substrate

2.6.4 Active Packaging Materials Containing Anti-microbial Systems

Recent developments in food preservation allowed the use of a wide range of antimicrobial compounds in food packaging materials to control food borne pathogenic and spoilage microorganisms (Ramos, Jiménez, Peltzer, & Garrigós, 2012). These antimicrobials can be of non-volatile or volatile nature. Non-volatile antimicrobials include bacteriocins, enzymes, organic acids, nano sized metal oxides and more recently bacteriophages. They have been incorporated directly into the polymer matrix to provide the packaging material with antimicrobial properties. On the other hand, volatile antimicrobials are easily released in a gaseous form to the headspace inside an enclosed packaging and can be incorporated directly into the polymer matrix as well as in sachets to develop antimicrobial packaging systems. According to (Pereira de Abreu, Rodriguez, & Cruz, 2012) the main advantage of using volatile antimicrobials is that there is no need of direct contact between food and packaging materials. Instead, the antimicrobial in a gaseous phase can reach almost any food matrix contained within the packaging system. In addition, the vapour has been proven to present higher antimicrobial activity than solution contact methods (Otoni *et al*., 2016)

Appendini & Hotchkiss (2002), reviewed an innovation in active packaging a potential for the controlled release of antimicrobials from packaging materials. Antimicrobials incorporated in packaging materials could extend shelf-life by preventing bacterial growth and spoilage. In one system, known as *"Bioswitch"*, an anti-microbial is released on command when bacterial growth occurs. The basic concept is that a change in the environment such as pH, temperature, or UV-

light occurs and the antimicrobial responds accordingly. The external stimulus results in a release of the antimicrobial component of the system only at specific conditions. This system could potentially increase the stability and specificity of preservation and reduce the amount of chemicals needed in foods. A common example of antimicrobial compounds in food packaging is the inclusion of polysacharide particles that encapsulate antimicrobial compounds. Many bacteria will digest polysacharide when they grow, so if a bacteria contamination occurs, the growth of bacteria will release the antimicrobial compounds and would inhibit subsequent microbial growth. Several works have been done where antimicrobials are added as active agents unto films to arrest microbial growth (Ramos, Jimnez, Peltzer, & Garrigis, 2012; Shojaee-Aliabadi *et al*., 2013; Wattananawinrat, Threepopnatkul, & Kulsetthanchalee, 2014). Results obtained from the investigation carried out by (Jouki, Mortazavi, Yazdi, Koocheki, & Khazaei, 2014) on the effect of quince seed mucilage edible films incorporated with the essential oils of oregano or thyme for shelf life extension of refrigerated trout fillets showed that the bacteria Pseudomonas spp. were significantly lower in samples wrapped with films containing combination of quince seed mucilage and 2% thyme than those exposed to the air.

It is noted that active packaging materials that can release active compounds for enhancing the quality and safety of a wide range of foods during extended storage are particularly important. This is because the release of active compounds plays an important role in determining the activity of the packaging material as well as in the inhibitory effect the packaging has on the spectrum of microorganisms.

2.7 Lipid Oxidation in Food

Lipids are important in foods because they contribute to food palatability, satiety, and nutrition. Lipid quality is also a very important issue to the consumers because poor quality lipid can ruin a whole lot of food in terms of taste and

texture. The nutrutional beneficial polyunsaturated lipids are minimally used in foods due to the fact that they are very suceptible to oxidation

Lipid oxidation is the series of chemical reaction that take place between acyl group of lipids and active oxygen. The unsaturated fatty acids on triacylglycerol's are relatively not volatile and do not directly give out flavours to foods, however during lipid oxidation these fatty acids decompose to form small, volatile molecules that produce the off-flavours associated with oxidative rancidity (Chaiyasit, Elias, Mcclements, & Decker, 2007)

There are two forms of rancidity in foods; hydrolytic and oxidative rancidity.

2.7.1 Hydrolytic Rancidity:

This form of rancidity occurs when fats and oils (lipids) come in contact with combination of enzymes and moisture in the presence of heat; Catalysing this reaction is lipase enzyme and heat while fatty acids and glycerol are liberated in the process. Such liberation of fatty acid is called hydrolysis, which is characterised with obnoxious odor, hence referred to as hydrolytic rancidity. In enzymatic reaction the enzyme (lipoxygenases) present within the oil matrix initiates the oxidation. (Antolovich *et al*., 2001)

2.7.2 Oxidative rancidity:

This is a non-enzymatic free radical-mediated chain reaction, which occur when unsaturated lipids exposed to air react with oxygen (auto oxidation). The process also ends up producing products with undesirable characteristics.

Photo- oxidation involves the reaction of singlet oxygen with lipids producing hydroperoxides without the formation of radicals. The oxidation mechanism is termed non-enzymatic, non-radical photo-oxidation; influenced by several factors such as light (photo-oxidation), heat, ionization and traces of metals,

Lipid oxidation generally is a major problem in many sectors of the food industry. Retarding lipid oxidation not only extends product's shelf life but also reduces

raw material waste, nutritional loss, and widens the range of lipids that can be used in specific products. Thus, control of lipid oxidation could allow food processors to use more available, less costly and more nutritionally favourable oils for product formulations (Dauqan, Abdullah, & Sani, 2011). Apart from producing offensive odors, oxidation of unsaturated lipids also end up destroying the essential nutritional quality and safety of foods due to their formation of secondary reactive (toxic) products. The process also produces dimers or polymers of lipids and proteins that reduce food quality (Eunok & David 2009). This flavor problem troubles a lot of vegetable oil industry and consumers inspite of the many advances made in processing and packaging technologies.

Lipid oxidation, expecially in bulk oil, is a homogeneous liquid phase reaction. Even even though edible oils is the presence of polar lipids, like free fatty acids,tocopherols sterols, phospholipids mono and diacylglycerols and some times small quantity of water. There is always some traces of these items even after refining process. When oxygen react with oils, it can also lead to the production of some polar oxidationproducts like lipid alcohols hydroperoxides, aldehydes, ketones, etc.The polarity of these new products arehigher than their original lipid substrates. This change is as a result of reaction with oxygen. Since polar lipids are surface active and thushave an affinity for both polar and non-polar environments, they tend to formassociated colloid structures (Wasowicz *et al*., 2004). A good understanding of the impact of surface active compounds on lipid oxidation in bulk oils led to the development of new antioxidative techniques to control rancidity, lipid oxidation rates and thus decrease shelf-life of the foods. The autoxidation rate greatly depends on the rate of fatty acid or acylglycerol, alkyl radical formation, while the radical formation rate depends mainly on the types of fatty acid or acylglycerol. The relative rate of autoxidation of oleate, linoleate, and linolenate is in the order of 1:12:25 on the basis of peroxide formation. Therefore, oils that contain relatively high proportions of polyunsaturated fatty acids (PUFA) may experience stability problems.

Lipid peroxidation referred to as the oxidative degradation of the membrane lipids leads to the products of hydroperoxides, such as alcohols, aldehydes, ketones, and alkanes which generally possess offensive off-flavors. Oxidation of essential fatty acids also produces toxic compounds and oxidized polymers. These compounds may also interact with other food components and change their functional and nutritional properties.

Furthermore, the oxidation of edible oils is influenced by an energy input such as light or heat composition of fatty acids, availability of oxygen, and minor compounds. Oxidation of lipids initiates other changes in the food system that affects its nutritional quality, wholesomeness, safety, colour, flavor and texture. Most molecules attacked by oxidation are unsaturated fats, causing them to go rancid and discolor. Fat rich foods are rarely preserved by drying; instead they are preserved by smoking, salting and fermenting (Onwuka, 2014).

Autoxidation is a spontaneous reaction of molecular oxygen with lipids, which leads to deterioration of food lipids. It is a free radical chain reaction. The overall mechanism of lipid oxidation involves three stage and discussed in terms of initiation, during which free radicals are formed; propagation, during which free radicals are converted into other radicals; and termination, during which two radicals combine to form stable products as shown in equations 2.3, 2.4 and 2.5

Initiation reaction

Homolytic hydrogen atom abstraction from a methylene group that leads to alkyl radical ($R^{\bullet}$) formation;

$$RH \rightarrow ROO^{\bullet} + R \qquad 2.3$$

Propagation reactions

The process entails the formation of peroxyl radicals (ROO•) able to react with unsaturated fatty acids and form hydroperoxides (ROOH);

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot\bullet} \qquad 2.4$$

Termination reactions

Formation of non-radical products by interaction of R• and ROO•.

$R^{\bullet} + R^{\bullet} \longrightarrow RR$

$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$ *2. 5*

Where: R• – fatty acid radical; ROOH – fatty acid hydroperoxide;

ROO•– peroxyl radical *(Chaiyasit et al., 2007).*

2.7.3 Free radicals

Free radicals are highly reactive molecules containing one or more unpaired electrons; they donate or take electrons from other molecules in an attempt to pair their elections and generate a more unstable species (Aliyu *et al*., 2012).The generation of primary free radicals is facilitated by the presence of oxidation initiators such as light, heat, ionizing radiation, transition metals, metalloproteins, oxidants, various homolysis-prone substances and enzymes (Senanayake, 2013). The unstable configuration in a free radical creates energy which is released through reactions with adjacent molecules, such as proteins, lipids, carbohydrates, and nucleic acids (Rahman, 2007). Due to their high instability they have the potential of harming human cells, and also damging biological molecules such as proteins, lipids or carbohydrates.

Oxygen-free radicals, are the major free radicals that destroy biological systems and they are known a “reactive oxygen species” (ROS).ROS are produced by chemical reaction like oxidation reaction, involving loss of electrons. Free radicals also combine with one another or with atoms that have unpaired electrons forming chain reaction Carocho & Ferreira (2013), reported that such free radicals when they react or interact with molecules, abstract a part to complete their own electronic structure, thereby generating new radicals, which

go on to react with other molecules. Such chain reaction are particularlly important in decomposition of substances at high temperatures.

Free radicals are also formed naturally in the body and play an important role in many normal cellular processes. When the body uses oxgen, it creats free radicals as by–products. Free radicals can also get accumulated in the body when highly oxidized foods (rancid foods) are taken or when the body is exposed to toxic environment, like cigarette smoke, ozone, and nitrous oxide, heat, ultraviolet light, and ionizing radiations all aid in generating free radicals. Free radicals steal electrons from cells, enzymes and cell membranes. Removing these electrons changes the composition and the structure it was stolen from. Cells are damaged and therefore do not function normally. At high concentrations, known as oxidative stress, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes (Carocho & Ferreira, 2013; Rahman, 2007). The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions (Lobo, Patil, Phatak, & Chandra, 2010).

Biological relevant free radicals are derived from oxygen and nitrogen and therefore called reactive oxygen specie (ROS) or reactive nitrogen specie (RNS). These elements are essential because they are converted to free radicals which are highly unstable, possessing highly reactive capacity that make them capable of damaging biologically relevant molecules such as proteins, lipid, or carbohydrate.

Hydroperoxides, tasteless and odourless compounds have been identified as primary products of autoxidation. The decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons and acids, which are known as secondary oxidation products of lipids. These products release offensive odour. The odour in terms of smell and taste is called “rancidity”. The process of rancidity not only affects the taste, smell and appearance but also reduces the nutritional quality of the food.

Reactive oxygen species can be classified into oxygen- centered radicals and oxygen-centered nonradicals. Oxygen- centered radicals are superoxide anion ($O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), alkoxyl radical ($RO^{\bullet}$) and peroxyl radical ($ROO^{\bullet}$), Oxygen centered non radicals are hydrogen peroxide (H_2O_2) and singlet oxygen (O_2)(Lobo *et al*., 2010).

Superoxide radicals ($O_2^{\bullet}$) an oxygen-cantered radical with selective reactivity is produced by a number of enzyme systems, by auto oxidation reactions, and by non- enzymatic electron transfers that univalently reduce molecular oxygen. In aqueous solution, $O_2^{\cdot}$can oxidize ascorbic acid. It can also reduce certain iron complexes such as cytochrome and ferric-ethylenediamine tetraacetic acid (Fe^{3+}-EDTA).

Hydrogen peroxide (H_2O_2)$^{\bullet}$ like HOCl and O_3, H_2O_2 is a free radical. It can cross membranes and may slowly oxidize a number of compounds. H_2O_2 can be formed *in vivo*. H_2O_2 at low (micromolar) levels also seems to be poorly reactive; however, higher levels of H_2O_2 can attack several cellular

Energy-producing systems; for example, it inactivates the glycolytic enzyme; glyceraldehyde-3-phosphate dehydrogenase. H_2O_2 also forms $OH^{\bullet}$ in the presence of transition metal ions, and O_2 can facilitate this reaction (Aruoma, 1998).

Hydroxyl radicals. **$(OH)^{\bullet}$** is a highly reactive oxygen-centered radical One feature of $OH^{\bullet}$ is that it begets another radical, i.e., when it reacts with a molecule, the result is the formation of another radical species. $OH^{\bullet}$ attacks all proteins, DNA, PUFA in membranes, and almost any biological molecule it touches.

2.7.4 Oxidative Stability

Oxidative stability of lipids is the resistance lipids to oxidation during processing and storage. The greater the level of unsaturation of oils the greater their rate of oxidation. Resistance to oxidation can be expressed as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change

or a sudden acceleration of the oxidative process (Nadaroglu, Demir, & Demir, 2007). It is an important parameter for the quality assessment of fats and oils and also determining their shelf life. This is due to the fact that low-molecular-weight off-flavor compounds are produced during oxidation and in trying to predict the stability of edible oils against oxidation stress, significant factors to be noted are the initial amount of the lipid free radicals and the quantity of inherent antioxidants in lipid (Choi & Awaji, 2005). The off-flavor compounds make oil less acceptable or unacceptable to consumers or for industrial use as a food ingredient. Oxidation stress in human body is known to be related to cause several pathological diseases like cardiovasscular, neurogenic, aging and even cancer. In mammalian cells, oxidative stress in humans arises from an imbalance in the antioxidant status (reactive oxygen species *versus* defence and repair mechanisms).

The prevention or retardation of these oxidation processes is essential for the food producer and for all persons involved in the value chain.

Methods of assessing antioxidant behaviour fall into two broad categories reflecting the focus on activity in foods or bioactivity in humans. In the case of food systems, the need is to assess the efficacy of an antioxidant(s) in providing protection for the food against oxidative spoilage. A subcategory involves measurement of activity in foods, particularly fruits, vegetables and beverages, but with a view to predicting dietary burden and *in vivo* activity

2.7.5 Measurement of Lipid oxidation

To analyse lipid oxidation in food products can sometimes be a hard task due to food complex nature, instability, large quantities of interfering substances in foods, and the sophistication and sometimes a lack of specific and adequate analytical methods (Wasowicz *et al*., 2004). The presence of natural antioxidants in food systems, being commonly the mixture of compounds of varying

mechanism of action, complicates the evaluation of the oxidative status of food lipids. Methods to determine the extent of oxidation can be ranked on the basis of their usefulness in predicting the stability, shelf-life and consumer acceptability. Still sensory analysis is given the closest approximation compared to consumers' approach. Methods such as chromatography which is based on the analysis of specific compounds, like chemical products of oxidation or even the extent by which oxygen is absorbed are useful in assessing the extent of oxidation. Some of the assessing mechanisms include peroxide value, p- anisidine value, trolox equivalent antioxidant activity (TEAC) value, Iodine value, free fatty acids (FFA), TBARS (mg MDA equivalents per kg of sample), hexanal count,etc.

(a) Peroxide value

The peroxide index or peroxide value is the most common parameter used to predict lipid oxidation. This parameter represents the total hydroperoxide and peroxide oxygen content of lipids or lipid-containing materials. It is commonly calculated from an iodometric titration developed over 60 years ago. This is the basis of current standard methods for determining peroxide value. In this method hydroperoxides and peroxides oxidize aqueous iodide to iodine which is then titrated with standard thiosulfate solution and starch as end-point indicator. The reactions and stoichiometries for this method are seen in equation 2.6 and 2.7

$$ROOH + 2H^+ + 2I_2 \longrightarrow I_2 + ROH + H_2O \quad (2.6)$$

$$ROOR + 2H^+ + 2I_2 \longrightarrow I_2 + 2ROH \quad (2.7)$$

Where ROOH is a lipid hydroperoxide and ROOR is a lipid peroxide. The PV is then calculated as milliequivalents of peroxide oxygen per kilogram of sample. Limitations involving this procedure are well recognized and include poor sensitivity and selectivity, possible addition of iodine across unsaturated bonds leading to low results, oxidation of iodide by dissolved oxygen and variations in reactivity of different peroxides. For these reasons other methods for determining peroxide oxygen have been investigated but the iodometric method still remains

the standard procedure. Peroxide value measures the primary oxidation products while the AV measures the secondary oxidation products and therefore points out the secondary stage of oxidation.

(b) Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay was proposed over 40 years ago and is now the most commonly used method to detect lipid oxidation. This procedure measures the malonaldehyde (MDA) formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate. It is postulated that the formation of MDA from fatty acids with less than three double bonds (*e.g.* Linoleic acid) occurs *via* the secondary oxidation of primary carbonyl compounds (*e.g.*, non-2-enal). The MDA is reacted with thiobarbituric acid (TBA) to form a pink pigment (TBARS) that is measured spectrophotometrically at its absorption maximum at 532–535 nm. Numerous substrates have been used in the determination of TBARS, including tissue samples, linoleic and other fatty acids and LDL used to characterize oils and fats (Wasowicz *et al.*, 2004). A product with peroxide value between 1 and 5 meq/kg is classified at low oxidation state; that between 5 and 10 meq/kg at moderate oxidation and above 10 meq/kg is classified at high oxidation state. However, Codex gives a peroxide value limit of 15 meq/kg for virgin oils in general

(c) The p-anisidine value *(p-AV)*

The chemical analysis method for P- Anisidine value determines the amount of aldehydes (principally 2- alkanals and 2, 4- dienals) in animal and vegetable oils and fats by reaction of these compounds with the p- Anisidine. This highlights the concentration of the quantity of aldehydes and ketones given the dimension of the secondary oxidation of the fat matrices.

(d) Spectrophotometric *Method:* The measurement of carbonyl content in the oils or fats, was determined by the standard method according to AOCS official

method Cd 18-90). It uses spectrophotometric analysis to measure the absorbance at 350nm. Based on the reactiveness of the aldehyde carbonyl bond on the p-anisidine amine group, leading to the formation of a Schiff base that absorbs at 350 nm.

In the analysis, p-anisidine (4- methoxyaniline) is used as a reagent to indicate the secondary stage of the oxidation, it is one of the three possible isomers of the anisidine or methoxyaniline. The other two isomers are o- Anisidine (2-methoxyaniline) and m-Anisidine (3- Methoxyaniline).

(e) Total Oxidation Value

(TOTOX) was used to estimate the oxidative deterioration of lipids. TOTOX value is defined as the sum of both values (PV and p-AV) to total oxidation and was calculated according to the formula in equation 2.8

TOTOX value = 2PV + p-AV (2.8)

2.8 Antioxidant (AO)

Antioxidants are compounds that delay the start or slow down the rate of lipid oxidation thereby increasing the shelf life of fats and oils and food products. They are often reducing agents such as thiols, ascorbic acid (vitamin C), vitamin E (α-tocopherol), carotenoids or polyphenols. They intercept and react with free radicals at a rate faster than their typical substrates. These free radicals have the ability to attack various targets, including lipids and proteins, and initiate oxidative reactions that result in the decreased shelf life of many foods. (Wasowicz *et a*l., 2004). Their addition to food substance is one of the most effective methods of retarding lipid oxidation even though they often occur in foods as native constituents of many fruits and vegetables, barks of trees and roots. Antioxidants are believed to play a very important role in body defence mechanism against reactive oxygen species (ROS). Naturally, there is balance between the amount of free radicals generated in the body and antioxidants to quench or scavenge them there by protecting the body against its deleterious effects. However, the quantity of these protective antioxidants present under the

normal physiological conditions are only sufficient enough to cope with the physiological rate of free- radical generation (Dauqan *et al*., 2011). But due to environmental pollution and consumption of foods, AO inhibit the formation of free radicals thereby preventing or delaying damage to the cells and tissues by assisting in terminating chain reactions produced by oxidation. In the process they are oxidized themselves and in so doing, AO contribute to the stabilization of the lipid food as well as other items that are affected by oxidation like polymer material. However, While some antioxidants are retarding lipid oxidation under one condition, in another condition they can be promoting lipid oxidation.(Huang, Ou,& Prior, 2005).

Antioxidants can deactivate radicals by two major mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET), depending on the kinetics and potential for side reactions (Huang, Ou, & Prior, 2005). HAT-based methods measure the ability of an antioxidant to quench free radicals by hydrogen donation. Reactivity in HAT methods is determined by the BDE (bond dissociation energy) of the H donating group in the potential antioxidant. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical and has been commonly used as one of the steps to screen phenolic compounds containing high free radical scavenging ability. When a hydrogen atom or electron was transferred to the odd electron in DPPH, the absorbance at 515–517 nm decreased proportionally to the increases of non-radical forms of DPPH. (Akinwunmi & Oyedapo, 2013; Stoilova, Krastanov, & Stoyanova, 2007). Conventionally, high free radical scavenging ability is regarded as high antioxidant activity and DPPH method has been used as one of the basic screening steps for searching for antioxidant compounds in organic solvent extracts. (Lee, Chung, Chang, & Lee, 2007). Another method is the Trolox method using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika, Rooney, & Waniska, (2004). This is a spectrophotometric technique and it measures the relative ability

of hydrogen-donating antioxidants to scavenge this $ABTS^{+}$ radical cation chromogen in relation to the water-soluble Vitamin E analogue, which is used as the antioxidant standard.

Electron donation of free radicals is represented in fig 2.2, where antioxidants would donate electrons to the thermaly induced free radicals in the packaged foods, thereby completing the outer shell ions of the radical species. This redox activity prolongs the shelf-life of the packaged food.

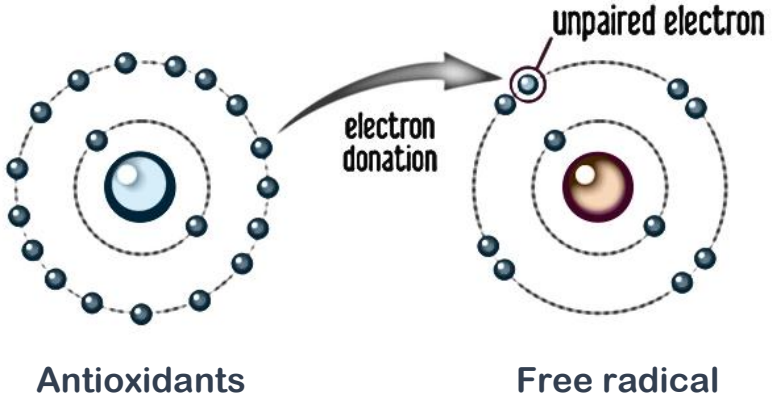


Figure 2. 2:*Electron Donation of Antioxidants to free radicals*

2.8.1 Classification of Antioxidants

Based on their mechanism of action Zuta & Simpson, (2007), reported that antioxidants are classified into primary and secondary, while based on their nature they are also divided into chemical and natural antioxidants.

(a) Primary antioxidants

Primary antioxidants (chain breaking antioxidants) are free radical scavengers that delay or inhibit the initiation step, or interrupt the propagation of auto-oxidation. Some of the well-known primary antioxidants in use in food products include tocopherols, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butyl hydroquinone (TBHQ).

(b) Secondary antioxidants

Secondary antioxidants slow down auto-oxidation by chelating prooxidant metals, furnish hydrogens to primary oxidants and decompose hydro peroxides

formed during the propagation step. They also deactivate singlet oxygen and absorb ultraviolet radiation. Secondary antioxidants may act synergistically with primary antioxidants to augment the latter is antioxidant activity. Examples of compounds that commonly act as synergists or secondary antioxidants include citric acid, lecithin, ascorbic acid and tartaric acid.

Antioxidants can play a preservative role through H-atom transfer in the lipid peroxidation Pathway as shown in equation 2.9 to 2.11

Initiation RH $\longrightarrow$ $R^{\bullet}$ (2.9)

Addition of $O_2R^{\bullet} + O_2$ $\longrightarrow$ $RO_2^{\bullet}$ (2.10)

H-atom exchange $RO_2^{\bullet} + RH$ $\longrightarrow$ ROO (2.11)

Reactions presented in equations 2.10 and 2.11 form a chain reaction when a free radical R• has been generated. Many lipid molecules (RH) are converted into lipid hydroperoxides (ROOH) as the chain cycles through reactions 2.10 and 2.11, resulting in oxidative rancidity of lipids. The reaction rate of 2.10 is very fast and occurs at about 10^9 $M^{-1}s^{-1}$, while the rate of 2.11 is much slower, generally 10^1 $M^{-1}s^{-1}$. Phenolic antioxidants (ArOH) contain at least one hydroxyl group attached to a benzene ring and has the role of interrupting the chain reaction as shown in equation 2.12.

$RO_2^{\bullet} + ArOH \longrightarrow ROOH + Ar\bullet$ (2.12)

The phenolic antioxidant must be a relatively stable free radical to be effective, so that it reacts slowly with substrate RH but rapidly with $RO_2^{\bullet}$

2.8.2 Synthetic antioxidants

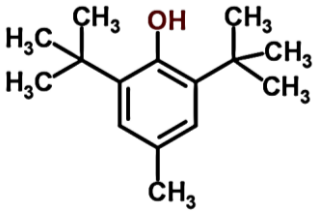
Synthetic antioxidants are synthesized monohydric or polyhydric phenols or phenolic structures with various ring substitutes such as polyphenol, organophosphate and thioester. Examples of the synthetic antioxidants include

tertiary butylated hydroquinone (TBHQ), butylated hydroxyanisole (BHA E320), butylated hydroxytoluene (BHT E321) and irganos 1010. These are monohydric or polyhydric phenols with various ring substitutes such as polyphenol, organophosphate and thioester. Nowadays, most food and pharmaceutical products contain these synthetic compounds to prolong product shelf life, mainly by preventing the oxidative deterioration and rancidity of unsaturated double bonds of fatty acids. The process is due to its lipid antiperoxidative activity. In North America the maximum allowable level of TBHQ is 0.02% with acceptable daily intake of 0-0.7mg/kg body weight. Studies have shown that chronic exposure to TBHQ may induce carcinogenicity, however its mechanism is not well understood (Gharavi *et al*., 2007). Some examples of the synthetic antioxidants include:

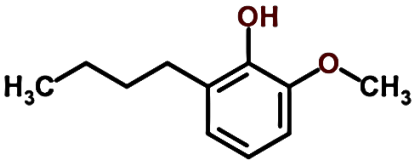
i. Antioxidant in the food industry. BHA can be easily applied to foods because of its excellent solubility in fats and oils. It is heat stable and of all antioxidants it has the best carry through effect into baked foods, providing extended shelf life. BHA can come in the form of powder, flake, tablet, small pack and liquid solution. Applications include: vegetable oil, animal feed, cereals, chewing gum, potato flakes and cosmetic products.
ii. Butylated hydroxytoluene (BHT) – is a synthetic analogue of vitamin E. just as BHA it operates by reducing oxygen radicals and interrupting the propagation of oxidation processes. Its volatility at higher temperatures makes it especially suitable for products that are a stored at moderate temperatures.
iii. Tertiary Butyl hydroquinone (TBHQ) – is a general purpose antioxidant used in many applications. Its strength increases with a higher degree of unsaturation, making it widely used in vegetable oils.
iv. Propyl gallate (PG) – is made from natural Garlic acid and shows excellent antioxidant activity in foods and vegetable oils, especially in combination

with ascorbyl palmitate. It is also synergistic with BHA. Propyl gallate shows lower solubility in oils compared to BHA and BHT.

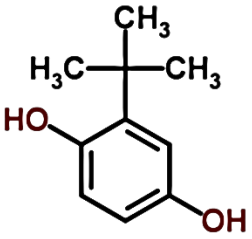
Chemical structures of natural antioxidants are related to those of syntheticantioxidants, and they work in a similar manner. Fig 2.3 gives the structures of some of the chemical antioxidants (Colon & Nerin, 2012)



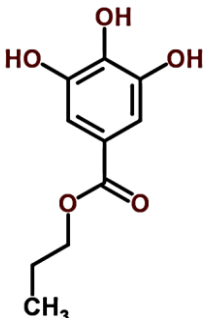
BHT ($C_{15}H_{24}O$)



BHA ($C_{11}H_{16}O_2$)



Tert-Butylhydroquinone (TBHQ) ($C_{10}H_{14}O_2$)



n- Propyl Gallate (PG) ($C_{10}H_{12}O_5$)

Fig 2.3 Chemical or synthetic Antioxidants

2.8.3 Natural Antioxidants (NAO)

A recent area of interest in antioxidant research is concerned with finding effective replacements for the conventional synthetic antioxidants from among various natural extracts from plant materials which are suspected to possess antioxidant properties. Natural antioxidants are constituents of many fruits and vegetables, tree backs and seeds. Oilseeds, spices and herbs, cereal crops, and crude plant drugs are potential sources of natural antioxidant compounds.

Vegetable oils in their natural form possess constituents naturally inherent in them that function as antioxidants. Some of this constituents are ascorbic acid, α-tocopherol, β- carotene, chromogenic acids and flavonoids (Grilo, *et al*., 2014). The smaller the presence of this inherent natural antioxidant the more they are prone to oxidation and vice versa.

These area of research is prompted by the reported possibility of synthetic antioxidants having adverse toxicological effect on humans; carcinogenic or liver enlargement and increased microsomal activity (Khanna *et al.,* 2002)

Extracts of seeds, leafs, stalk of plants containing natural antioxidants have been reported to exert favourable effects on human health like protection against cardiovascular diseases, anti-inflammatory activity and anti-carcinogenic effects and this is as a result of their phenolic contents (Khanna, *et al.,* 2002; Shrikhande, 2000)

Most of the isolated constituents from plants with antioxidant (AO) activities are phenolic compounds. Rosmary (*Rosmarinus officinalis*) which is a member of the Lamiaceae family. Its extract is one of the plant extracts that has already been reported to possess high antioxidant activity (Bolumar, LaPeña, Skibsted, & Orlien, 2016). It is composed of flavones (Apigenin, genkwanin, hesperetin and cirsimaritin), phenolic diterpenes (carnosic acid, carnosol, rosmadial, epirrosmanol, rosmanol, carnosic acid o-quinone), and phenolic acids (caffeic acid and rosmarinic acid). Carnosic acid is one of the most important compounds responsible for AO capacity. Green tea also has potent AO capacity since it is a good source of polyphenolic compounds like catechin, theaflavins and thearubigins, which have the ability to scavenge reactive oxygen and nitrogen species (Siripatrawan & Noipha, 2012). Therefore, green tea extract has already been used successfully to prepare active packaging (Colon & Nerin, 2012) and active edible films (Siripatrawan & Noipha, 2012). Grape fruit seed extract is

made from seeds and pulp of grapefruit and it contains tocopherol, citric and ascorbic acids. The AO and antimicrobial effects of this extract in different products such as ground beef have been reported (Song *et al.,* 2012).

There has been intense efforts in search of natural antioxidant compounds that are not related to possible toxicological side-effects, especially those that could be carcinogenic (George & Osioma, 2011). This is because of consumers' preference for natural antioxidant as against the synthetic antioxidants with perceived health risk.

Natural antioxidants have been used nowadays to stabilise edible oils from oxidation, It has been used to supplement the naturally inherent antioxidant present in the oils. Some fatty foods like olive oil are patially protected from oxidation by their natural content of antioxidants but they still remain sensitive to photooxidation (Yanishlieva & Marinova, 2001). Test conducted to investigate the effectiveness of natural antioxidants contained in red pepper oil added to soybean and sunflower oils indicate that they provide variable protection against light induced auto-oxidation. Measuring fatty acid and peroxide value formation indicate the inhibitive effect on oxidation.

In another study on the inhibitive action of tocopherol on rape seed and palmkernel oils by measuring the presence of the oxidative product monoaldehyde, indicate some measure of protection provided by these natural antioxidants.

2.8.3.1 Vitamin E (tocopherols and tocotrienols):

Vitamin E is a generic term used for several naturally occurring tocopherols and tocotrienols. It is one of the most important lipid-soluble primary defence antioxidants. In its function as a chain-breaking antioxidant (Abdalla, 2009). Vitamin E rapidly transfers its phenolic H-atom to a lipid peroxyl radical,

converting it into a lipid hydro peroxide and a vitamin E radical (Bashir, Guido, Wim, & Aalt, 2004). Tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS. Out of four isomers of tocopherols (α-, γ-, β-, δ-) found in plants, a-tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure.

Palm vitamin E (30% tocopherols, 70% tocotrienols) has been extensively researched for its nutritional and health properties. The tocotrienols have been reported to be natural inhibitors of cholesterol synthesis (Abdalla, 2009). Tocopherols (vitamin E) and tocotrienols (provitamin E) are powerful antioxidants that confer oxidative stability to red palm olein as well as help to keep the carotenoids and other quality parameters of the oil stable. Vitamin E scavenges peroxyl radical intermediates in lipid peroxidation and responsible for protecting poly unsaturated fatty Acid (PUFA) present in cell membrane and low density lipoprotein (LDL), against lipid peroxidation. Tocopherols, a lipid soluble antioxidant are considered as potential scavengers of ROS and lipid radicals. Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activities through scavenging oxygen radicals and some times metal chelators (Nwakaudu *et al*,. 2015). Some examples of common plant phenolic antioxidants include flavonoid compounds of cinnamic derivatives such as coumarins, tocopherols and polyfunctional organic acids, examples are apigenin, genkwamin,hesparetin, and cirsimaritin. Phenolic diterpenes (carnosic acid, carnosol, rosmadial, epirrosmanol, rosmanol, carnosic acid o-quinone), and phenolic acids (caffeic acid and rosmarinic acid) and Carvacrol. Many natural free radical scavengers such as catechins (catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate) and Quercetin also have health benefits as inhibitors of biologically harmful oxidation reactions in the body. They are known as non- volatile flavonoids. (Shrikhande, 2000)

Several studies have been carried out in order to identify natural phenolics that possess antioxidant activity. They and some of their derivatives are very efficient in preventing auto oxidation. (Womeni, Djikeng, Tiencheu, & Linder, 2013; Yanishlieva, Marinova, & Pokorný, 2006). The antioxidant activity of these phenolic compounds are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quencher (Sofidiya *et al.*, 2006). Vitamin E is a generic term used for several naturally occurring tocopherols and tocotrienols. It is well known for its role as an antioxidant, and it is a powerful biological antioxidant. It is one of the most important lipid-soluble primary defence antioxidants (Abdalla, 2009). Antioxidants such as vitamin E act to protect cells against the effects of oxidative stresses induced by free radicals. The phenomenon called oxidative stress results when an overload of free radicals cannot gradually be destroyed as a results of accumulation in the body. This can generate a series of damaged cells and may contribute to the development of cardiovascular disease, cancer, tissue injury in liver, brain, kidney, lung, nervous system and other organs (El-shebly, 2009; Halliwell & Cross, 1994). In its function, as a chain-breaking antioxidant, vitamin E rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydro-peroxide and a vitamin E radical. Ascorbic acid (Vitamin C) and Ascorbates are water soluble antioxidants. They are used in fruit products such as juices, jams, and canned drinks, acidic and fatty foods. Ascorbates prevent fruit juices from descoloration. Structures of some natural antioxidants are illustrated in fig 2.4.

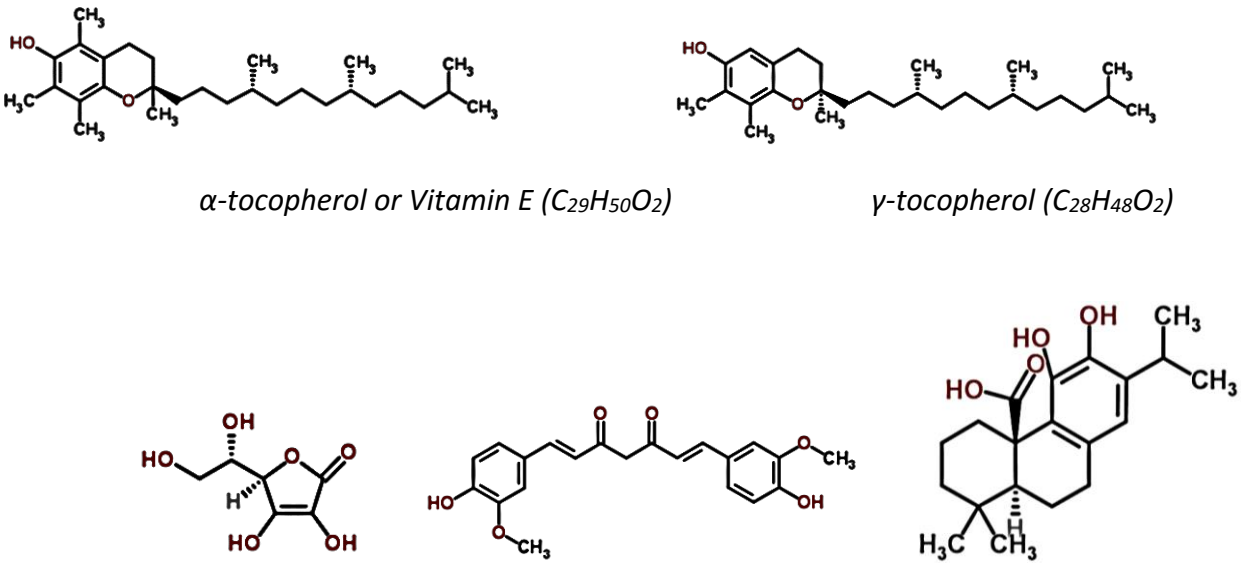


Fig 2.4 Natural Antioxidants

2.8.3.2 Spices and Herbs

Spices are a large group of natural aromatic substances which include dried seeds, fruits, roots, rhizomes, barks, leaves, flowers and any other vegetative substances used in a very small quantity as food additives to colour, flavour or preserve food (Brit., 2006) Spices are fragrant, aromatic and pleasant. Spices are abundant sources of polyphenolic compounds which have strong antioxidant capacities and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits. Consumption of spices has been associated with the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis (George & Osioma, 2011). The bulk of the spices consist of carbohydrates such as cellulose, starch, pentosans and mucilage and some amount of protein and minerals (Odukoya, Inya-Agha, Segun, Sofidiyaand, & Ilori, 2007). Only very small portions of dry matter of the spices such as the phytochemicals are responsible for the flavouring, colouring, preservative and health-promoting characteristics (Sofidiya et al., 2006).

These phytochemicals are plant metabolites which act as natural defence systems for host plants, and also provide characteristic colour, aroma and flavour in specific plant parts. They are a group of non-nutrient compounds that are biologically active when consumed by human (Dhan & Girish, 2014). Many phytochemicals are health promoting and many others disease-preventive. Typical phytochemicals with antioxidant activities include polyphenols, phenolic acids and their derivatives, flavonoids, phospholipids, ascorbic acid, carotenoids and sterols (Leonov *et al*., 2015)

A lot of exotic spices of international recognition such as chilli pepper, garlic, cinnamon, etc. (Brit., 2006), whose phytochemical constituents are known have been proven to be good natural antioxidants.

African nutmeg is a perennial edible plant of the Annanacea family that grows wild in the evergreen forests of West Africa. Its local names include; Ehuru (Igbo), Ariwo (Yoruba), Jamaica nutmeg and Ariana (Ekeanyanwu , Ugu, & Nwachukwu, 2010). Spices have also been used as preservatives to suppress microbial activities. For these reasons several researches have been carried out on the assessment of the antioxidant activity of many herbs, spices and their extracts when added in different foods and food systems. A lot of consideration has been given to herbs of the Lamiaceae family, particularly oregano and rosemary, These herbs have been reported to have great antioxidant activities (Camo *et al*., 2011; Sánchez-Escalante *et al*., 2001).

Herbs and spices have been used for many centuries to improve the sensory characteristics and to extend the shelf-life of foods. (Camo *et al*., 2011). Diferent spices possess different and unique aroma or flavour. Spices possess this capacity due to the presence of phytochemicals inherent in them andthey can also be used to mask other flavors. They are usually rich in essential oils and are used either in fresh or dry form. Spices tend to add few calories to food, even

though many spices especially those made from seeds, contain high portions of fat, protein, and carbohydrate by weight. Many spices however, can contribute significant portions of micronutrients (e.g. Vitamin A, iron, magnesium, calcium, etc.) to the diet. Nigeria has several indegenous spices resources namely: *Aframonum longiscarpum (K.Schum), Allium cepa L, and A. Sativum L, Anona senegalensis Pers, Arachis hypogeal L, Asystasia gagentica (T. Anders), Capiscum annum L and C. frutescens L, Cymbopogon critrates L, Diociecia reflexa, Gnetum africanum, Gongronema latifolium, K eayodendron brideliode, Mondora myristica, ocimum reflexa, Gnetum africanum, Gongronema latifolium, K gratissimum L, Parkia bigslobose L, Xylopia aethiopica,* among others . More recently, the interest in spices and herbs has grown not only for their seasoning and flavoring properties, but also for their antioxidant potentials (Baiano, 2018; Odukoya et al., 2007; Sofidiya et al., 2006). Many herbs, spices, and their extracts have been reported as having high antioxidant capacity (Almasaudi *et al*., 2016; George & Osioma, 2011; Stoilova *et al*., 2007) such as some plants of the Lamiaceae family, oregano (*Origanum vulgare L.*), and ginger. (*Zingiber officinale*), rosemary (*Rosmarinus officinalis L.*), sage (*Salvia officinalis L.*). Spice extractives, such as oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavor in some products. Several studies have demonstrated that some edible plants or vegetable diets possess substantial antioxidant properties. The antioxidant activity of these plants is attributed to their total phenolic compound content (Velasco & Williams, 2011). Polyphenols, phenolic compounds, flavonoids, and terpenes are well known for their antioxidant activity (Apak *et al*., 2007; Khalaf *et al.,* 2008; Tátraaljai *et al*., 2013). Phenolic compounds from edible plants are the main antioxidants in the human diet (Ghasemzadeh, Jaafar, & Rahmat, 2010). There is a growing interest in the antioxidant and anti- inflammatory capacities of these compounds relative to prevention or treatment of chronic diseases that involve inflammation (Wang *et al.,* 2011). For example, dietary phenolics have been

related to reduced risk of cancer by protecting cells from damage induced by oxidative stress and cardiovascular disease (Visioli & Hagen, 2007).

The United States Food and Drug Administration (FDA) defined spices as aromatic vegetative substances used for seasoning of food and from which no portion of any volatile oil or flavouring principles have been removed, and are free from artificial colouring matters, adulterants and impurities. Spices are "Generally Regarded as Safe" (GRAS) by the FDA, at least at concentrations commonly found in foods. Spices have less nutritional value, and they cannot be grouped as food. The major components of spice materials consist of carbohydrate, protein, and little minerals. Tannins, resins, pigments, volatile, essential and fixed oils which contribute to flavouring occur in traces and constitute only a small fraction of the dry matter. Some examples of spices include chilli pepper, turmeric, garlic, ginger, nutmeg, clove, cinnamon, etc. Spices are characterized by pungency, strong odour, sweet or bitter taste. Spices in food exert some secondary effects such as salt and sugar reduction, prevention of spoilage and improvement of texture (Visioli & Hagen, 2007).

Besides imparting flavour to foods and drinks, spices also stimulate appetite, increase the secretion and flow of gastric juices and help digestion because of their carminative properties (Pilgrim, *et al* 2015). For this reason, they are commonly known as 'food Adjuncts' or 'food Accessories'. Most of them are used in different medicines. They also play an important part in various industries and are used in perfumery, soaps, incense, dyes, etc.

Some spices and derivatives possess antioxidant and antibiotic properties, which have increased interest in the commercial exploitation of aromatic plants for food preservation and crop protection. With the growing demand of natural and organic products and the increasing clamour to dispense with synthetic flavours and artificial food colouring, the future for spices seem bright. Spices because of

the health-promoting phytochemicals they contain are known to fight cancer and many heart diseases. The flavouring, preservative and antiseptic properties of some of these spices are primarily due to the presence of volatile oils, but are occasionally due to other aromatic substances.

2.9 Phytochemicals

Phytochemicals literally "plant chemicals" are a large group of plant bioactive compounds which amazingly, are responsible for much of disease protection. They are commonly found in fruits, vegetables, nuts, legumes and grains. Phytochemicals also act as natural defence system for the host plant. Plant use phytochemicals as natural protection from bacteria, fungi and viruses (Altemimi, 2017). These phytochemicals which are part of a large and varied group of chemical compounds are also responsible for the colour, flavour, and odour of plant foods such as blueberries dark hue, broccoli's bitter taste, onion and garlic's pungent flavour and also the burning sensation of hot pepper. More than 4000 of these compounds have been discovered and it is expected that scientists will yet discover many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs and spices (Dhan & Girish, 2014).

Phytochemicals can have profound physiological effects, act as antioxidants, mimic body hormones and suppress development of diseases in the body. Examples of this are isoflavones found in soy and the lignins found in flax. These can mimic oestrogen in the body (Lecomte, *et al*., 2017). Researchers have reported that phytochemicals have the potential to stimulate the immune system, prevent toxic substances in the diet from becoming carcinogenic, reduce inflammation, prevent DNA damage and aid DNA repair, reduce oxidative damage to cells, trigger damaged cells to self-destruct (apoptosis) before they can reproduce, help regulate intracellular signalling of hormones and gene expression, and activate insulin receptors. (Leonov *et al*., 2015).

One of the major functions of phytochemicals is their role as antioxidants. Consuming foods rich in phytochemicals can prevent diseases such as cardiovascular disease, cancer, type 2 diabetes, and neuron degeneration. (Van der Schouw*et al.*, 2000). Common phytochemicals are resveratrol in grapes/grape skins, Isoflavones in Soy, Lycopene in tomatoes, Lutein in spinach and Naringenin in grapefruit etc.

2.9.1 Classes of Major Phytochemicals and Food Sources

Phytochemicals also referred to as phytonutrients are numerous and are found in all plant products, including fruits, vegetables, legumes, cereals, herbs and spices (American Institute of Cancer Research, 2000) and are classified according to their chemical structures and functional properties. The terminology used to describe phytochemicals (flavonoids, flavonols, flavonones, proanthocyanidins, procyanidins) can be confusing. Phytochemicals include compounds such as salicylates, phytosterols, saponins, glucosinolates, polyphenols, protease inhibitors, monoterpenes, phytoestrogens, sulphides, terpenes, lectins and many more (Kaminski *et al*., 2012). Fig 2.5 gives the phytochemical family tree that shows the major groups of phytochemicals found in food.

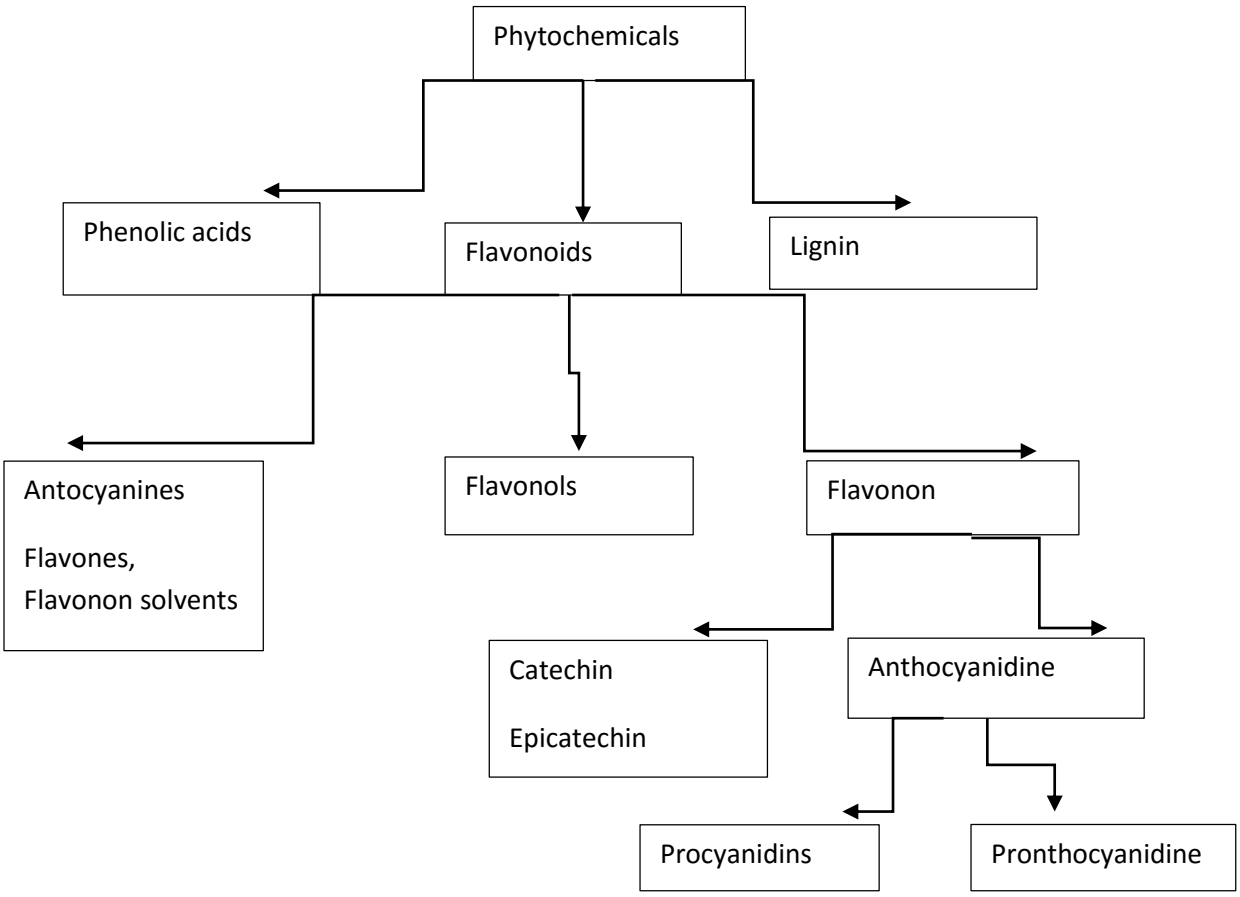


Fig 2.5: Phytochemical family tree.

— *Source: Heneman K, Zidenberg-Cherr S. Some facts about phytochemicals. UC Cooperative Extension Center for Health and Nutrition Research Nutrition and Health Info Sheet.* http://nutrition.ucdavis.edu/content/infosheets/fact-prophytochemical.

Some good food sources of phytochemicals include cabbage, lettuces, tomatoes, carrot, water melon, mangoes, pawpaw, grapes, oranges, apples, cashew apple and nut, mustard, pears, oats, sweet potatoes, whole wheat, beans, ginger, onions, red pepper, spinach, sesame seed and garlic among others (Hayes, 2005). According to Brit., (2006), phytochemicals work in synergy and their effects when served together are stronger than the sum of the effects of parts served separately.

2.9.2 Phytochemical Metabolism in Human

Most phytochemicals found in foods exist in a variety of forms which influence their digestion and absorption. Most common ones are the polyphenols which exist as glycoside conjugates. Some glycosides must be digested to glycones (unconjugated forms) before being absorbed. Some other forms of phytochemicals are thought to be absorbed in the intestines without intensive digestion. The absorption of most phytochemicals is thought to involve a carrier. Also, many glycosides are neither digested nor absorbed in the small intestine. Such phytochemicals not absorbed in the small intestine have been shown to undergo microbial degradation by colonic micro flora. The bacteria hydrolyse the glycosides, generating a glycones which may undergo further metabolism to form various aromatic compounds. Once absorbed, most phytochemical metabolites get conjugated in the small intestine or in the liver. Conjugation most often involves methylation, sulfating or glucumidation. These conjugated metabolites are then bound to plasma proteins such as albumin and are transported through the blood to various parts of the body. The amount of these conjugated metabolites in the plasma varies considerably with the type of polyphenol consumed, the food source, and the amount ingested. However, after consumption of specific polyphenols, little is known about the metabolism of the different polyphenols in the body, and also about what metabolites are present in the plasma.

2.9.2.1Polyphenols

Polyphenols, a large class of chemicals which are found in plants, have attracted much attention in the last decades due to their properties and the hope that they will show beneficial health effects, when taken as dietary inputs or as complements. Phenolic compounds constitute one of the most extensive groups of phytochemicals in the plant kingdom. It is estimated that more than 8000 compounds have been isolated and described as Polyphenols are

polyhydroxylated phytochemicals, which have common structures. (Carlsen *et al.*, 2010) Polyphenols are present in many edible plants; they are thought to be their presence, along with that of other molecules such as carotenoids, vitamin C or vitamin E that is responsible for the health effects of fruits and vegetables. In the human diet, they are the most abundant natural antioxidants. Foods rich in polyphenols especially fruits and vegetables translate into benefit on human health (Dai & Mumper, 2010). Polyphenols are potent antioxidants, they are able to scavenge free radicals. Among polyphenols, there are simple molecules, such as phenolic acids, or complex structures such as condensed tannins, that are highly polymerized molecules. Polyphenols can be classified into different classes according to the number of phenolic rings in their structure, the structural elements that bind those rings and the substituents linked to the rings. Therefore, two main groups can be identified; the flavonoid group and the non-flavonoid group. Phenolic acid which is., a sub-division of the non-flavonoid group account for about one third of the total intake of polyphenols in human diet. These compounds are capable of removing free radicals and inhibiting oxidases as a result, they neutralize free radicals formed during normal physiological functioning of human body (Oboh., 2006). The antioxidant activity of phenol is due to their redox properties through which they act as hydrogen atoms or electron donors, singlet oxygen quenchers, reducing, metal chelating agents and their ability to scavenge free radicals (Amarowicz *et al*., 2004).

2.9.2.2 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in green plants cells. They are the largest, most varied and most studied groups of phytochemicals. In fact, more than 6000 flavonoids that occur in plant foods have been described (Selamoglu, 2017). Flavonoids also referred to as bioflavonoids, as natural occurring biological compounds that are often found in plants. Flavonoids are

characterized as containing two or more aromatic rings, each bearing one or more phenolic hydroxyl groups, and connected by a carbon bridge

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. They belong to the polyphenol family and can be visualized as two benzene rings joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids are made up of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids (Hussein, 2017). Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables and herbs. Quercetin, kemferol, catechin and epigallo-catechin-3-gallate (EGCG) are examples of flavonoids. They are actually a type of antioxidants that act as secondary metabolites. The oxidation of low-density lipoprotein (LDL) has been recognized to play an important role in atherosclerosis. Many studies have confirmed that one or two glasses of red wine daily can protect against heart disease.
Tea flavonoids have many health benefits as they reduce the oxidation of low-density lipoprotein, lowers the blood levels of cholesterol and triglycerides. Soy flavonoids (isoflavones) can also reduce blood cholesterol and help to prevent osteoporosis. Soy flavonoids are also used to ease menoposal symptoms (Zheng, Lee and Chun, 2016). Immune system cells called macrophages recognize and engulf oxidized LDL; a process that leads to the formation of atherosclerosis plays in the arterial wall. LDL oxidation can be induced by macrophages and can also be catalysed by metal ions like copper. Several studies have shown that certain flavonoids can protect LDL from being oxidized. They do so by lipid peroxidation of scavenging reactive oxygen/nitrogen species, chelation of transition metal ions and sparing of LDL-associated

antioxidants.There are so many benefits associated with partaking in a diet that is rich in flavonoids. A few of the health benefits include:-

a) A reduced risk of cancer due to the powerful way they help the body build immunity and fight off unhealthy scavengers. Flavonoids have also been found to help fend off the onset of cardiovascular diseases and may help prevent related consequences such as heart attack or stroke(Peterson *et al.*, 2012; Tangney & Rasmussen, 2013). The anti-inflammatory properties of flavonoids also help the body fight off the worst effects of allergies.

b) Flavonoids have also been reported to inhibit the formation of blood clots, which also prevents the onset of heart attack and strokes(Sebastian *et al.*, 2017; Tang, *et al.*,2016)

c) Recent studies have shown that flavonoids have properties which also prevent ulcers. Almasaudi *et al*. (2016), in their research with Manuka honey which is a honey rich with flavonoids reported out that the flavonoids were able to prevent gastric ulcers in ethanol-induced gastric ulcer rats. Flavonoids have been demonstrated to have anti-inflammatory, anti-allergenic, anti-aging and anti-carcinogenic activities (Liu *et al*., 2017; Pandey *et al*., 2016; Pérez-Cano & Castell, 2016).

d) Flavonoids may help provide against these diseases by contributing along with antioxidant vitamins and enzymes to the total antioxidant defence system of the human body. Flavonoids, a group of naturally occurring benzo-g-pyronederivatives, have been shown to possess several biological properties including; hepatoprotective, anti-thrombotic, anti-inflammatory & anti-viral activities. These may be related partially to their antioxidant and free radical scavenging abilities. The anti-radical property of flavonoids is directed mostly towards HO, and O_2 – as well as peroxyl and alkoxyl radicals. Furthermore, as these compounds present a strong affinity for iron (which are known to catalyse many processes leading to the

appearance of free radicals), their anti peroxidative activities could also be ascribed to a concomitant capability of chelating iron (Huyut, Beydemir, & Gülçin, 2017).

2.10 African nutmeg "Ehuru" (*Monodora myristica*)

This is an aromatic seed spice belonging to a tropical tree of the family Annonaceae or custard apple family of flowering plants. Ehuru is a light-brown or greyish wrinkled seed inside a smooth hard blackish brown nut. The nut is always dried in the sun until the inner seed rattles when shaken.In the olden days, its seeds were widely sold as an inexpensive nutmeg substitute but nowadays, it is less common outside its region of production. Other names of African nutmeg include Jamaican nutmeg, Calabash nutmeg, ariwo, awerewa ehiri or Ehuru, airama, African orchid nutmeg, muscadier de calabash and lubushi (Akinwunmi & Oyedapo, 2013). The fruit contains numerous pale brown aromatic seeds embedded in a yellow pulp. Each seeds meaures about 1.5cm in diameter.The African nutmeg tree grows naturally in evergreen forests from Liberia to Nigeria and Cameroon, Angola and also Uganda and West Kenya (Eze-Steven, Ishiwu, Udedi, & Ogeneh , 2013). The odor and taste of the *Monodora myristica* seed is similar to Nutmeg and it is used as a popular spice in eastern part of Nigeria and West African cuisines in general. The fruits are collected from wild trees and seeds are dried and sold whole or ground to be used in stews, soups, cakes and desserts. The high lipid content is indicative of the fact that these spices are good sources of flavour since they are rich in essential oil and also suggests possible sources of oil - soluble vitamins. Enwereuzoh *et al*., (2015), reported that the essential oil obtained from the leaves of ehuru contain β-caryophyllene, , α-humulene and α-pinene. Nutmeg has 6.5% to 16% essential oil, which is pale yellow in colour and is called oil of *Myristica*. Phytochemical screening carried out on *Monodora myristica* extract by Feyisayo & Oluokun (2013), revealed the presence of Tannin, saponin, flavonoid, steroid, terpenoids, cardiac glycoside,

alkaloid and phenol. Earlier determination of the chemical constituents of the seeds revealed the presence of Fiberro-latic oils, resins, terpene, lactose, arocine, saponins, flavonoids and tannins. Depending on the source, the major compounds found in the essential oil are mainly sabinene (15-50%), α-pinene (10-22%) and β-pinene (7-18%), with myrcene (0.7-3%), safrole (0.1-3.2%) and terpinene (0-11%), cineole (1.5-3.5%), *Myristicin* (0.5-13.5%), Limonene (2.7-4.1%) (Feyisayo & Oluokun, 2013). The fixed oil is a pale yellow viscous oil, and 6 g of Oleoresin is equivalent to 100 g of freshly ground nutmeg. Nwozo, Kasumu, & Oyinloye (2015) in their research reported that Monodora *myristica* has the ability to lower blood cholesterol, as it lowered total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) in the serum, while it helped in increasing high-density lipoprotein cholesterol (HDL-C) in the serum. High level of LDL-C has been shown to be one of the risk factors for the development of atherosclerosis and related cardiovascular diseases in human (Gertz & Reardon 2006). Report from Okonkwo & Ogu (2014), showed that *Monodora myristica* possess some preservatives and anti-oxidant properties while on diabetes George & Osioma, (2011) reported that nutmeg has insulin-like activity. Fig. 2.6 and 2.7 show the ehuru seeds. The flavonoid fraction of *Monodora myristica*is is shown to posses signifiicant anti-inflammatory potentential; by stabilizing red blood cell membrane exposed to hypotonic and heat induced lyses with maximum percentage stability (Akinwunmi & Oyedapo, 2015).

Fig 2.6: Decorticated Ehuru seeds

Fig 2.7: Undecorticated Ehuru seeds

2.11 α-tocopherol as a Chain Breaking Antioxidant

α-tocopherol (Figure 2.4) is a chain-breaking antioxidant that reacts with peroxyl radicals with much faster rate constant than the reaction of peroxyl

radicals with lipid RH (Wright, Johnson, & DiLabio, 2001). The bond dissociation enthalpy (BDE) of phenolic antioxidants is an important factor in determining the antioxidant effectiveness, since the reaction rate with free radicals is faster with weak OH bonds. The $RO_2^{\bullet}$ radical has a BDE on formation of the parent ROOH of about 88 kcal/mol, which will react rapidly in an exothermic reaction with α-tocopherol. The BDE of α-tocopherol is about 76 kcal/mol, while β-, γ-, and δ-tocopherol have slightly higher respective BDEs of 78, 78, and 80 kcal/mol (Wright *et al*., 2001). These BDEs enable the tocopherols to function as effective chain-breaking antioxidants that prevent lipid peroxidation. The rate constant for the hydrogen atom transfer from α-tocopherol to cumyl peroxyl radicals at 25°C decreases by approximately 2 orders of magnitude upon transitioning from hexane to ethyl acetate as solvent (Muchuweti *et al*., 2007). This solvent effect may be explained in terms of hydrogen bonding between α- tocopherol, which acts as hydrogen donor, and the solvent, which acts as hydrogen acceptor (Pedrielli, Pedulli, & Skibsted, 2001). The more solvating solvent induces added stability to the reactants relative to the transition state, which effectively increases the activation energy of the reaction. Most vegetable oils contain tocopherols with the more unsaturated oils having higher concentrations of up to 1000 mg/kg or greater (Evans, Kodali, & Addis, 2002). In the more saturated vegetable oils, such as coconut and palm kernel, tocopherols are almost completely lacking. Several types of tocopherols exist, such as α-, β-, γ-, and δ-forms, which differ from one another in the position and number of methyl groups on the phenol ring. A series of corresponding tocotrienols also exist in which the 16-carbon side chain is unsaturated. During the storage of unsaturated oils, tocopherols are consumed and their concentrations fall as oxidation proceeds. α-tocopherol exhibits the highest antioxidant activity of the tocopherols in vegetable oil with the least stability during storage (Prochazkova, Bousova, & Wilhelmova, 2011). The degradation rate of α-tocopherol for 10 days of storage in soybean oil was 5.6% per day. α-tocopherol degraded faster than

both γ- and δ-tocopherol with its degradation rate 10 times faster than δ-tocopherol. α-tocopherol easily donates a hydrogen atom to the peroxyl radical due to its relatively low BDE and is the most easily destroyed. α- tocopherol is expected to function as a more potent hydrogen donor than γ- or δ-tocopherol due to its fully methylated structure. The reported stability of α-tocopherol varies widely among researchers and appears strongly dependent upon factors of atmosphere, sample matrix, and analytical method. The stability of pure α-tocopherol upon storage was approximately halved with each 10°C increase in temperature. The half-life of pure α-tocopherol at a temperature of 40 °C was 113 days, while at 120 °C the half-life was reduced to only 0.9 days. The degradation of α-tocopherol followed an induction type of curve with α-tocopherol having less stability in its pure form than when diluted in an oxidatively stable solvent. A complete loss of α-tocopherol is observed upon storage of red palm oil, groundnut oil, and their mixed blends (Lakshmi, Narmadha, & Reddy, 2008). α-tocopherol is highly unstable to heat in red palm oil with 89% losses due to heating at 130 °C for 15 min during a frying process. Significant decreases in tocopherols also occurred during the extrusion process of fish- and peanut containing half-products. Imran *et al.,* (2015) reported decreases in α-tocopherol content in fish and peanut extrudate to be 23 and 18% respectively under extrusion conditions of between 100°C and 250 rpm these differences in α-tocopherol content are attributed to differences in fatty acid composition of the raw materials. Oxidative degradation of α-tocopherol has been studied by micro calorimetry and is clearly a temperature-dependent process between 50–80 °C (O'Reilly, Sanders, & Wiseman, 2000).

2.12 Quercetin

Flavonoids are a group of aromatic secondary plant metabolites that belong to a class of phenolic substances. The major classes of flavonoids include flavones, flavonols, flavanones, isoflavones, anthocyanidins, and cathecins. Quercetin

(Fig. 2.4) is the most common flavonol found in the human diet and epidemiological studies suggest it protects against cardiovascular disease (Sebastian *et al.*, 2017). The predominant sources of quercetin in the diet include onions, apples, tea, and berries. Quercetin has been reported to be among the most effective of the flavonoid antioxidants and the most active in its own flavonol class (Zheng, Deng, & Lai, 2017).

The antioxidant activity of quercetin has been evaluated in terms of chemical structure-activity relationships. Flavonoids with the same basic structure, such as quercetin, rutin, and apigenin, have peroxyl radical scavenging activity that increases with the number of –OH substituents. Three structural determinants are proposed to be responsible for effective radical scavenging by flavonoids: (1) the ortho-catechol group in the B-ring, which gives high stability to the radical formed; (2) the conjugation of the B-ring to the 4- oxo group by the 2,3-double bond that ensures electron delocalization from the B-ring; and (3) the 3- and 5-OH groups with the 4-oxo group, which allows electrons to be delocalized from the 4-oxo group to both substituents. These structural features function together to enable a higher electron delocalization which gives a higher stability to aroxyl radicals (Kuzuo, Ouchi, Nakaua, & Shin-chi Nagaoka, 2013).

Quercetin is a potent antioxidant in oil systems due to its free radical scavenging activity, UV-absorbing characteristics, and iron-chelating capacity. Quercetin shows greater antioxidant effectiveness against UV-induced lipid oxidation in an oil emulsion than BHT (butylated hydroxytoluene). Pedrielli et al., (2001) reported that Quercetin did not have significant antioxidant activity in sunflower oil at 60 °C in the presence of endogenous tocopherols and added citric acid however, a significant antioxidant effect is found with the presence of tocopherols alone. This indicates that quercetin is active as a metal-chelating agent in this system. On the other hand, Zuta, Simpson, Zhao, & Leclerc, (2007) observed

that quercetin exerts strong antioxidant effects in fish oil and a synergistic effect when combined with α-tocopherol while its oxidative degradation was observed to cause unusual changes in antioxidant capacity, which they observed to be both temperature and solvent dependent.

Different solvent systems appear to select the specific reaction pathway that is followed. In ethanol or methanol, oxidative reactions result in the formation of complex polymers, which lead to an initial increase and then subsequent decrease in antiradical activity. In hydro-alcoholic solutions, a decrease in antiradical activity is observed during quercetin degradation. The polymerization of quercetin may be prevented by the presence of water with its high hydrogen-accepting capability, which makes oxidative cleavage the most probable reaction pathway. Quercetin experiences degradative losses of approximately 83% in 0.2 M KOH solution at pH 13.0 and 97 °C within 30min. Degradation of quercetin under these strong alkaline conditions occurs at a slower rate at 20 °C, but results in nearly complete decomposition within 250 min. Oxidative conditions convert flavonols into compounds that are sensitive to degradation even at ambient temperature. The presence of oxygen in these quercetin solutions greatly accelerated its degradation rate with complete degradation occurring within only a 40 min period at 25 °C

2.13 Prooxidant action of an antioxidant

Antioxidants may exhibit prooxidant behaviour dependent upon the specific conditions of the system, particularly antioxidant concentration level, sample matrix, and the presence of transition metals. Prooxidant activity can accelerate damage to lipids, proteins, carbohydrates, pigments, and vitamins. Most of the phenolic acids, flavonoids, anthocyanidins, and anthocyanins exhibit some prooxidant activity at low concentrations, while this is not observed with α-tocopherol. Prochazkova *et al.*, (2011) observed that quercetin prooxidant activity increases with increasing concentration until the antioxidant activity of the

compound rapidly becomes dominant at about 200 μM. The prooxidant effect of flavonoids is most pronounced at low antioxidant concentrations (<1–2 μM), but is reversed at higher concentrations and masked by dominant inhibition of initiation. In the presence of Cu_2^+, Medini & Abdelly, (2014) observed that flavonoids acted as prooxidants rather than antioxidants and this activity always increased with flavonoid concentration in the range they studied; up to 2 μM. It was further reported that this copper-initiated prooxidant activity of flavonoids is dependent upon the number of –OH substitutions in the flavonoid structure. Tocopherol in bulk vegetable oils is observed to experience antioxidant activity inversion with its greatest activity at lower concentrations and either decreases or even prooxidant activity at higher concentrations. The prooxidant effects of α-tocopherol observed at high concentrations are not well understood. At high concentrations, α-tocopherol inhibits hydroperoxide decomposition but promotes hydroperoxide formation (Tafazoli, Wright, & O'Brien, 2005). The optimal concentration for α-tocopherol to exhibit greatest antioxidant potency in soybean oil is in the range of 340 and 660ppm. Peroxide values of α-tocopherol increase as the α-tocopherol concentration increases from 100 to 1000 ppm with significantly higher values at 500 ppm indicating prooxidant activity (Evans *etal*., 2002). α-tocopherol functions as the most potent of the tocopherols in soybean oil at preventing oxidation as measured by conjugated diene formation. The maximum antioxidant activity of α-tocopherol in stripped corn oil was determined to be 100 ppm on the basis of hydroperoxide formation. Low concentrations of α-tocopherol in the 50–100 ppm range are optimal to minimize oxidation of mackerel oil with higher concentrations proving less effective. Oxidized tocopherols are indicated to act as prooxidants in soybean oil, with oxidized α-tocopherol exhibiting a stronger effect than oxidized γ- or δ-tocopherol (Evans *et al*., 2002).

2.14 Review of Previous and Related Research Works on Natural Antioxidant (NAO) Used In Active Packaging

Natural antioxidants (NAOs) can be used in the packaging with the aim of having an action on the packaging which is either stabilizing the package against UV degradation or (2) with the intention of being released into the food in oder to stabilize the food against oxidation. Therefore in the first case, NAOs would respect packaging legislation (Yam, 2009) and, in the second case, it would respect the food legislation (Community or national provisions) (Regulation (EC) 450/2009).

2.14.1 Pure standards of natural AOs

Tocopherols are classified as substances generally recognised as safe for food products according to the Code of Federal Regulations (FDA 2013). According to Siró *et al.*, (2006) α-tocopherol is one of the most used AOs to protect the polymer during processing and as an additive for active packaging. Granda-Restrepo et al.(2009) have developed a multilayer active packaging with high-density polyethylene (HDPE), ethylene vinyl alcohol copolymer (EVOH) and an internal layer of low-density polyethylene (LDPE) in which the LDPE layer incorporates α-tocopherol. This new packaging has delayed the lipid oxidation of milk powder. Noronha *et al*., (2014) in their work developedand characterised an active film made from the addition of a-tocopherol nanocapsules in a methyl cellulose film. Their study revealed that α-tocopherol nano capsule (NCs) demonstrated high antioxidant activity and excellent barrier property against UV and visible light.

Sanches-Silva *et al.*,(2014) reported that the first natural antioxidant incorporated into synthetic polymers was α-tocopherol (Vitamin E). Wessling *et al.*, (2000) reseached on antioxidant ability of BHT and alpha tocopherol impregnated LDPE film in packaging of oat meal reported that the active package loses its activity

faster due to faster migration of the active components added.They also reported that the rate of migration was reduced by using a film layer that has low diffusion of the antioxidants or through the use of cyclodextrin. Jongjareonrak, Benjakul, Visessanguan, & Tanaka (2008), in their investigation of the oxidative activity and properties of fish skin gelatin films incorporated with BHT and α –tocopherol observed that incorporation of α-tocopherol in polymers has been widely investigated, but not much in biopolymer.

Siró *et al.*, (2007) reported that α- tocopherol exhibits an excellent melt stabilization to polyethylene and polypropylene at very low concentrations in the range of 100–300 ppm, which surpasses that of the stabilization provided by some of the best synthetic hindered phenol antioxidants traditionally used for this purpose. Therefore, they recommended that a NAO like α-tocopherol can be employed cost-effectively for polyolefin stabilization. In comparism to the synthetic antioxidant, Torres-Arreola *et al., (*2007) reported that α-tocopherol to be a superior antioxidant for reducing off-odor and off-taste from high density polyethylene (HPDE) bottles compared to the typical synthetic antioxidants, Irganox 1010 and BHT.

Arrieta *et al.*, (2014) investigated plasticizing polylactic acid (PLA) a bio polymer using limonene, a natural antioxidant intended for food packaging application. The presence of D-limonene improved the crystallinity and flexibility of the film.

Ma, Ren, & Wang(2017) investigated the antioxidant activity and release kinetcs of curcumin from the active film. In their work, active film was developed by incoprating curcumin in a bio polymer made up of combination of polyvinyl alcohol and taragum. The study showed that the rate of curcumine diffusion coefficient increased with increasing curcumin content. Also, higher temperature accelerated curcumin diffusion.

2.14.2 Antioxidants from Cereals

Barley husks were used to prepare an active food packaging due to their composition in phenolic compounds. Barley is one of the most important cereals cultivated worldwide. Some researchers also reported sorghum and millet to be a good source of phenolic and antioxidant compounds with a variety of genetically dependent types and levels including phenolic acids, flavonoids, and condensed tannins which could be incorporated into a package (Awika *et al.*, 2004; Dykes & Rooney, 2006; Sikwese & Duodu, 2007). These grain are used in the brewing process and the spent grains containing the husk has very little use apart from fibre for feed stocks. Pereira de Abreu *et al.*,(2010), reported that, after pre-hydrolysis and delignification of barley husks, it is possible to obtain a rich AO extract. The main phenolic compounds of this extract are p-coumaric and ferulic acid. Dykes & Rooney (2006) reported that the antioxidant level in barley husk is comparable to that of flavonoid quecetin and catechin.

2.14.3 Antioxidants from Crustaceans

Research work carried out by Sanches-Silva *et al.,* (2013) reported the incorporation of a potent AO, astaxanthin, obtained from shrimp by-products in an LDPE matrix. These by-products were fermented and the silage was centrifuged, given three phases: a solid phase (from which chitin is obtained), a liquid phase with proteins, minerals and free amino acids, and a lipid phase that contains astaxanthin. Astaxanthin is a carotenoid with an AO activity 100–500 times higher than β-carotene and vitamin E, respectively. Moreover, this compound is responsible for the pink-red colour of salmonids and shellfish.

The AO capacity of food is influenced by its composition / nature, the packaging film type and the atmosphere in the inner inside of the packaging (Bentayeb *et al.,* 2007). The AO properties of the film depend on the AOs incorporated, the thickness of the film and the nominal concentration of the AOs in the film.

Bentayeb *et al.*, (2007) also reported that even though the AO capacity increases with the concentration of the incorporated AOs, but the increase is not linear. For higher concentrations the slope is smoother probably due to an interaction between the AOs and the film.

These differences on the mechanical behaviour of films with EAE agree with reports from a number of the following reseachers: Ma, Ren, & Wang, (2017) prepared antioxidant film from curcumin and noticed that tensile stress decreased with curcumin addition, were the elongation at break of the film increased. Martinez-Pardo, Shanks, Adhikari, & Adhikari, (2017), also observed that a-tocopherol incorporation significantly decreased TS of chitosan films ($p < 0.05$), while Siripatrawan & Harte, (2010) reported that the incorporation green tea extract in chitosan-based films caused a significant reduction in tensile strength when compared to film control. Noronha, De Carvalho, Lino, & Barreto, (2014a), also reported a significant TS decrease on fish skin gelatine films incorporated with a-tocopherol, where the elongation at break significantly decreased with the addition of nanocapsules (NCs) when compared to that of control films ($p < 0.05$).

2.14.4 Natural Antioxidants active film from Spices

The target interest now is on antioxidants naturally present in spices and herbs due to their lower toxicity and higher safety. In line with this natural sources of antioxidant such as leafy spice extracts of green tea *(Camellia sineensis L.)* Rosemary (*Rosmarinus officinalis L.*), extracts of oregano also known as *"thyme"* (*Origanum vulgare L.)* others like curcumin (turmeric), ginger, Ehuru (Monodora *myristica*) (Akinwunmi & Oyedapo, 2013; Camo *et al.*, 2011; Pereira de Abreu, Paseiro, Maroto, & Cruz, 2010; Tátraaljai *et al.*, 2013) lemon (Vilela *et al.*, 2013) and extracts of sage (*Salvia officinalis L.*) had been used as active agents that have been incorporated into polymer food packaging.

Differentpure standards of natural (AOs) have also been incorporated into food packaging materials. Baschieri, Ajvazi, Tonfack, Valgimigli, & Luca Amorati, (2017) reported the high antioxidant activity of some non phenolic components of essential oils like limonene and linalool. α-tocopherol, (Arrieta *et al*., 2013; D. Liu et al., 2016) caffeic acid, catechin (López-de-Dicastillo, *et al.*, 2012; López-de-Dicastillo *et al.*, 2010), quercetin (López-de-Dicastillo *et al.*, 2010; Gomez-Estaca *et al.*, 2009).

2.14.4.1 Antioxidants from Ehuru (Monodora *myristica*)

In a study by George & Osioma (2011) the antioxidant properties of local spices including ehuru were investigated. Result showed that ehuru was evaluated as potential source of natural antioxidant. Similarly**,** the result obtained from a study conducted by Akinwunmi & Oyedapo (2013) on the antioxidant potentials of *Monodora myristica* suggested that flavonoid fraction of *M. myristica* seed possessed significant antioxidant properties. On the other hand, (Enwereuzoh et al.) 2015 extracted the flavonoids from ehuru and used it to season popcorn showing that flavouring agents can be obtained from ehuru. Nwozo *et al*., (2015) also evaluated the cholesterol lowering potential and protective ability of aqueous extract of Monodor*a myristica.* The study suggested that *M. myristica* possesses cholesterol lowering potentials and protective ability.

All the available works were limited to identification of antioxidant properties of ehuru or its being a veritable source of natural antioxidant no attempt has been made to incorporate it into polymeric materials to produce active package films.

2.15 Measurement of antioxidants

Antioxidant activity can be calculated as a percentage inhibition of one or more of either the primary or secondary products of oxidation in relation to the control (Antolovich et al., 2001). A variety of methods are being used in measuring the antioxidant activity depending on the mechanism adopted whether hydrogen atom transfer (HAT) or sequential electron transfer (SET).

2.15.1 DPPH scavenging Assay.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a radical scavenging mechanism that makes use of Oxygen radical absorbance capacity (ORAC); one important antioxidant method utilizing HAT reaction mechanisms. ORAC measures antioxidant inhibition of peroxyl radical-induced oxidations and thus reflects classical radical chain breaking antioxidant activity by H atom transfer (Bentayeb *et al.*, 2007). 1,1- diphenl-2-picrylhydrazyl (DPPH) method of evaluation of free radical – scavenging activity of foods is an example. (DPPH) is a molecule that is distinguished for its stable free radical which is due to the delocalisation of the spare electron over the molecule as a whole, in other not to make the molecule dimerize, like most other free radicals would do. The product of this delocalization of electron is a deep violet colour, characterized by an absorption band in ethanol solution at about 517 nm.

A solution of DPPH when mixed with a substrate (AH) that can donate a hydrogen atom H, results in its reduced form accompanied with loss of the violet colour. Fig. 2.9 illustrates this mechanism.

Diphenyl-2-picrylhydrazyl radical

Diphenyl-2-picrylhydrazyl (Nonradical)

Fig 2.9: DPPH Scavenging Mechanism

Among free radical scavenging methods, DPPH method is more rapid and simple (i.e. does not involve too many steps and reagents) and inexpensive in comparison to other test models

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to Jouki, Yazdi, Mortazavi, & Koocheki, (2014) the sample extract

(0.2 m*l*) is diluted with methanol and 2 m*l* of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using equation 2.13.

% inhibition of DPPH radical = ((A_{br} - A_{ar})/ A_{br}) x100 2.13

Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

2.15.2 ABTS radical cation decolourization assay

$ABTS^{+}$ (2, 2-azino-bis (3-ethylbenzthiazoline- 6-sulfonic acid) is a stable radical having a blue green colour. When using it to measure antioxidant activity a spectrophotometer to measure its colour loss as an antioxidant is added to the $ABTS^{+\cdot}$ The antioxidant reduces $ABTS^{+}$ to ABTS and decolourizes it. ABTS radical cations are prepared by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of ABTS (20 mL using a 75 mM Na/K buffer of pH 7) as described by Alam & Bristi (2013).

2.15.3 Hydrogen peroxide scavenging (H_2O_2) assay

Alam & Bristi (2013), in their review of invivo and invitro methods of antioxidant evaluation reviewed that the ability of plant extracts to scavenge hydrogen peroxide can be estimated using a solution of hydrogen peroxide (40 mM) prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophoto meter. Extract (20–60 g/m*l*) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated by using equation 2.14.

% scavenged H_2O_2 = [(A_i-A_t)/ A_i] x 100 2.14

2.15.4 Nitric oxide scavenging activity

Sodium nitroprusside (SNP) compound is known to decompose at neutral pH, in aqueous solution to give NO^- which reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent. 2 m*l* of 10 mM sodium nitroprusside dissolved in 0.5 m*l* phosphate buffer saline (pH 7.4) is mixed with 0.5 m*l* of sample at various concentrations (0.2–0.8 mg/m*l*). The mixture is then incubated at 25 ^{O}C. After 150 min of incubation, 0.5 m*l* of the incubated solution is withdrawn and mixed with 0.5 m*l* of Griess reagent [(1.0 m*l* sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1m*l* of naphthyl ethylenediamine dichloride (0.1% w/v). The mixture is then incubated at room temperature for 30 min and its absorbance is measured at 546 nm. The amount of nitric oxide radical inhibition is calculated using equation 2.15

$$\% \text{ inhibition of NO radical} = [(A_0 - A_1)/ A_0] \times 100 \qquad 2.15$$

Where A_0 is the absorbance before reaction and A_1 is the absorbance after reaction has taken place with Griess reagent.

2.15.5 Reducing power method (RP)

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a coloured complex with potassium ferric cyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. In the method described by Henderson, Nigam & Owusu-Apenten (2015) 2.5 m*l* of 0.2 M phosphate buffer (pH 6.6) and 2.5 m*l* of K_2Fe_3 (CN)6 (1% w/v) are added to 1.0 m*l* of sample dissolved in distilled water. The resulting mixture is incubated at 50 ^{O}C for 20 min, followed by the addition of 2.5 m*l* of Trichloro acetic acid (10% w/v). The mixture is centrifuged at 3000

rpm for 10 min to collect the upper layer of the solution (2.5 m*l*), mixed with distilled water (2.5 m*l*) and 0.5 m*l* of $FeCl_3$ (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample.

2.15.5.1 Ferric reducing-antioxidant power (FRAP) assay

This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the complex of ferric iron and 2, 3, 5-triphenyl-1, 3, 4-triaza-2-azoniacyclopenta-1, 4-diene chloride (TPTZ) to the ferrous form at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer. Antioxidant assay can be conducted by the method of Benzie, Szeto, & Africa (2000). Three millilitre of prepared FRAP reagent is mixed with 100 m*l* of diluted sample; the absorbance at 593 nm is recorded after a 30 min incubation at 37^{o}C. FRAP values can be obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mM of Fe^{2+} equivalents per kg (solid food) or per litre (beverages) of sample.

Ferric Reducing Antioxidant Power (FRAP) assay, is One of the most useful methods of SET (Electron transfer). It measures reduction of (TPTZ) to a coloured product (Antolovich *et al*., 2001). SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyl and radicals. Relative reactivity in SET methods is based primarily on deprotonation and ionization potential (IP) of the reactive functional group.

2.15.6 Hydroxyl radical scavenging activity

Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. The scavenging ability of hydroxyl radicals is measured by the method of Benzie & Szeto (1999). The mixture consists of 100 m*l* of 2-deoxy-D-ribose (28 mM in 20 mM KH_2PO_4-KOH buffer,

pH 7.4), 500 ml of the extract, 200 m*l* EDTA (1.04 mM) and 200 mMFeCl3 (1:1 v/v), 100 m*l* of H_2O_2 (1.0 mM) and 100 m*l* ascorbic acid (1.0 mM) which is incubated at 37 ^{O}C for 1 h. One millilitre of thiobarbituric acid (1%) and 1.0 m*l* of trichloro acetic acid (2.8%) are added and incubated at 100 ^{O}C for 20 min. After cooling, absorbance is measured at 532 nm, against a blank sample.

Lipid peroxidation is therefore a major cause of food deterioration and this leads to a loss of functional properties and nutritional values (Tian *et al*., 2013). Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis. The major pathway of lipid peroxidation contains a self-catalytic free radical chain reaction. However, lipid peroxidation can be catalysed by environmental factors, such as light, oxygen, free radicals and metal. The oxidation of lipids in food leads to a reduction in shelf-life due to changes in taste and/or odour, deterioration of the texture and functionality of muscle foods, and a reduction in nutritional quality (Pereira de Abreu *et al.*, 2010). Unlike bacterial or fungal spoilage, spoilage of food due to oxidation reactions still occur relatively in frozen or refrigerated environment (Vigili & Morino, 2008).

Fat rich foods are rarely preserved by drying; instead they are preserved by smoking, salting and fermenting. Importantly they are preserved in an oxygen free package. Exposure to oxygen and sunlight are two main factors that aid oxidation of food. Chemical antioxidants like BHA and BHT food additives (preservatives) are used to prevent fatty foods from going rancid when exposed to oxygen. They are added in baked products, cereals, potato chips, and fats and oils. Tocopherols (vitamin E) are used to prevent rancidity in fats and other damage to food exposure to oxygen (Onwuka, 2014).

2.16 Antioxidants as Polymer Anti-degradants

Antioxidants are also used to prevent degradation of the polymer as a result of its reaction with atmospheric O_2, during processing operations at high temperature or when used in contact with hot foods. They are also used to prevent brittleness

during storage (Al-Malaika & Kong, 2005). Many unsaturated polymers can undergo degradation from a range of sources, such as oxygen absorption, which leads to the release of organic peroxides. To reduce the risk of mechanical and chemical deterioration, phenolic or amine antioxidants can be added to these polymers. Some of the common additive groups used for these purposes include: Derivatives of phenols and organic sulphides most frequently used is Hindered phenol**.** A phenol consists of a benzene ring coupled with a hydroxyl group. When adjacent carbon atoms have their bonded hydrogen replaced with heavier elements, the result is a hindered phenol compound, which prevents oxidative degradation of polymers such as rubber.They are radical scavengers which prevent thermal degradation of many organics and polymeric materials. Its effectiveness increases in combination with phosphites, thioethers and light stabilizers (Till *et al*., 1982). Other additives used in polymer industry include plasticizers

2.17 Plasticizers in bio polymers

Plasticizers are important class of low molecular weight non-volatile compounds that are widely used in polymer industries as additives. In biopolymer-based films and coatings production, plasticizers are also essential additives since they can improve flexibility and handling of films, maintain integrity and avoid pores and cracks in the polymeric matrix. (Gurgel *et al*., 2011). Plasticizers are, in general, high boiling point liquids with average molecular weights of between 300 and 600, and linear or cyclic carbon chains (14–40 carbons).

Arrieta et al.,(2014) and Noronha et al., (2014) in their work reported that natural additives like limonene improves the plasticizing and crystallinity of polymer films. The low molecular size of a plasticizer allows it to occupy intermolecular spaces between polymer chains, reducing secondary forces among them. In the same way, these molecules change the three-dimensional molecular organization of polymers, reducing the energy required for molecular motion and the formation of hydrogen bonding between the chains. This consequently leads to

an increase in the free volume and, hence, in the molecular mobility as observed. The degree of plasticity of polymers is largely dependent on the chemical structure of the plasticizer, including chemical composition, molecular weight and functional groups (Ma, Yu, & Wan, 2006). Therefore, they soften polymers by lowering the glass transition temperature (Tg) and thus, reducing elastic modulus.

2.18 Method used in producing Active Polymer film

There are three main methods used in producing active polymer film/package:extrution, cast, and coating methods.

2.18.1 Active Polymer Using Coating Process Method

Currently used strategies for protecting foodstuff and the like include coating oxygen sensitive materials with antioxidant compositions, coating the antioxidants themselves with substances that allow for sustained release, and mixing antioxidants with carriers such as polymers (Bastarrachea, Wong, Roman, Lin, & Goddard, 2015). However, existing methods and compositions typically allow the oxygen scavenging material to leach out of its carrier into the oxygen sensitive materials.

2.18.2 Active Polymer Using Solvent Casting Method

Solvent casting method is mostly used to produce bio polymer films. Nowadays the solvent cast technology is becoming increasingly attractive for the production of films with extremely high quality requirements. The advantages of this technology include uniform thickness distribution, maximum optical purity and extremely low haze. The optical orientation is virtually isotropic and the films have excellent flatness and dimensional stability. The key elements of cast film manufacturing are for dope preparation, die design, casting support, film drying and solvent recovery if necessary. The process makes use of solution blending method; In the case of lab-scale practice, solution blending method is commonly used for preparation of polymer/active component. This method includes three basic steps, namely: dissolving the polymer with a suitable solvent, dispersion of

active component in solvent and mixing with the dissolved polymer, and finally casting on a solid plate. In solution blending method most of polyolefin polymer, such as Polyethylene (PE) and Polypropylene (PP), are hardly soluble in common organic solvents, therefore elevated temperature is applied.

It should be noted here that cast films can in most cases be re-dissolved and reused as raw material. The solution or polymer film is exposed to relatively low thermal or mechanical stress throughout the entire production process. As a result, degradation or adverse side reactions are insignificant. Biodegradable films are mostly produced by cast method. Noronha *et al*., (2014) produced an antioxidant methylcellulose film incorporated with a-tocopherol nanocapsules. Fig 2.10 illustrates the cast method.

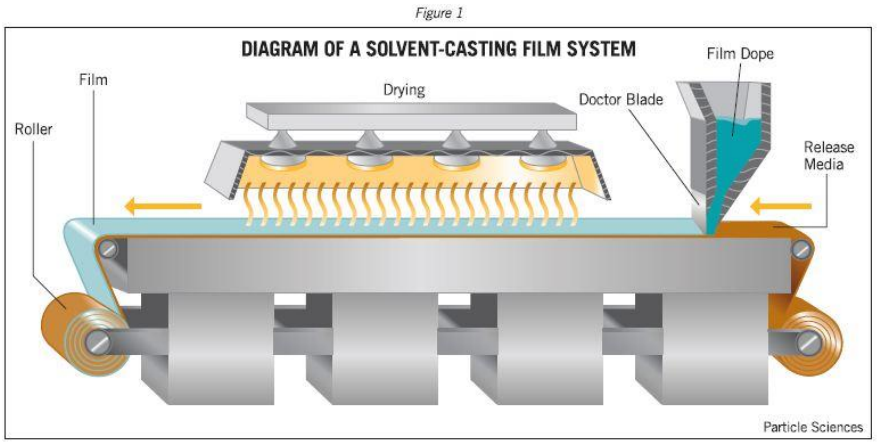


Fig 2.10 Diagram of solvent Casting film system

2.18.3 Active Polymer Using film Extrusion Method

An extruder is classified into three: single screw, twin-screw, and multiple screw extruders. Twin-screw extruder can be adapted easily to process various requirements and product characteristics.

Basically, the principal mechanism of twin-screw extruder is thermal processing with high heat, high pressure, and involvement of shear forces in the process. The benefits of using this extruder are that it is low cost, applying speed, having high productivity, and its versatility. In general, there are seven zones in twin-screw

extrusion process; solid feeding, melting, filler feeding, dispersing, homogenizing, degassing, and discharging (Anggoro, 2014). The zonation of twin-screw extruder is an example depicted in Figure 2.11. Each zone represents each process and it has its own function, starting from input material, conveying, mixing, distributing, dispersing, etc. It links from one to another, thus it can be considered as individual process. Since it employs screws, the arrangement of the screws is also important. It may influence the performance of extrusion such as product transformation, residence time distribution, and mechanical energy input.

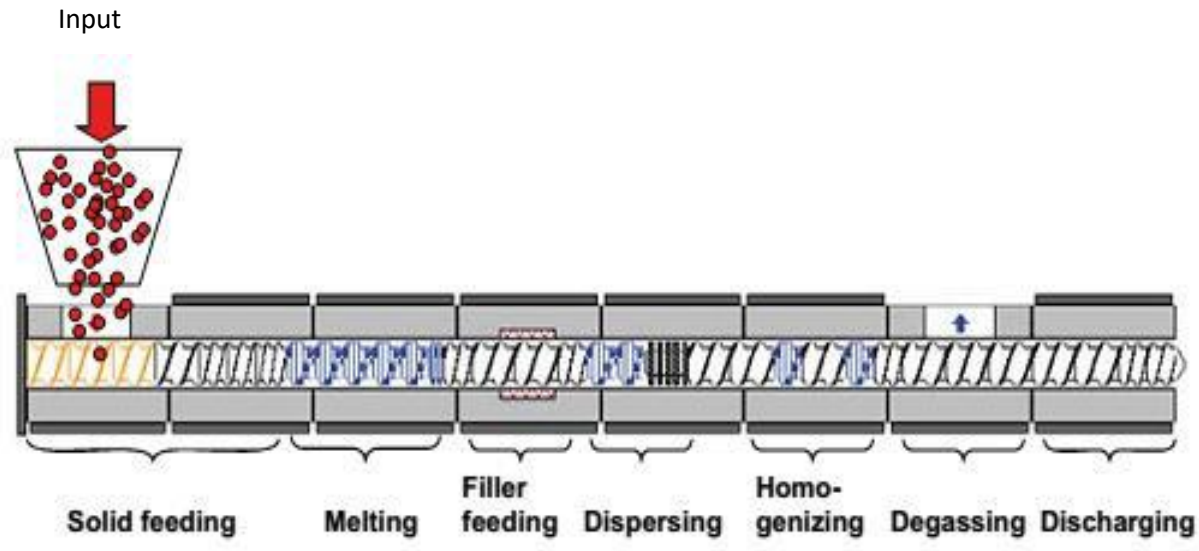


Figure 2.11. Zonation twin-screw extruder
Source:Anggoro, (2014)- openaccess.iyte.edu.tr

Thermoplastic Polymer materials are commonly processed into several forms using the extrusion process. When used to produce active package there is the draw back of low efficiency of most of the developed materials in in *vivo* tests and the degradation of active agents in the extrusion processes. This is due to the fact that extrusion occurs at very high temperature of over 180^{o}C.The extrusion film process can be categorised into *"tubular film"* production, sheet extrusion, and Flat film or *"slit-die"* extrusion.

i. T***ubular film***: This process involves forcing out a molten plastic material through a die and blowing the molten material up into a bubble of several diameters more than the die diameter. This makes it possible for a biaxial extension of the molten material and thining of the plastic film. Figure 2.12 shows an illustration of the tubular extrusion process.

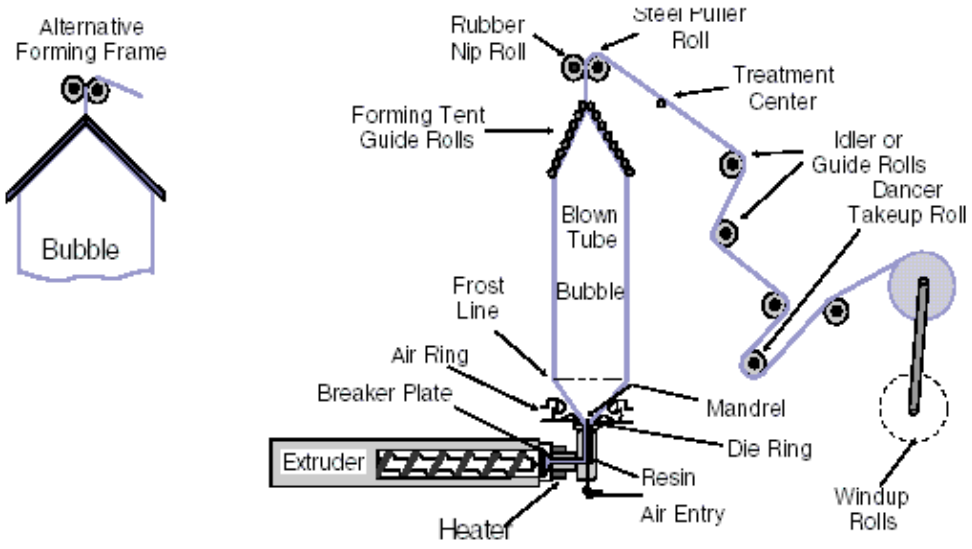


Figure 2.12: T*ubular* extrusion blown film process

ii. **Sheet extrusion.** This process involves the extrusion of molten plastic through a slit die. The material is then allowed to fall directly into a cooling bath or unto a chill-roll assembly where it is rapidly cooled. The resulting sheet is calendered and drawn through a series of rollers to the required thickness. Finally, the sheet is collected on winding rolls and packed.

iii. Flat film or *"slit-die"* extrusion. This is similar to sheet extrusion but with an increase in the speed of the take-up rolls at slightly elevated temperatures. This drastically reduces the thickness of the film to the required thickness. In most cases, the film resulting therefrom is only oriented uniaxially, in the direction of the take-up rolls.

The following researchers used extrusion method in forming their active films (Arrieta, Fortunati, Dominici, López, & Kenny, 2015; Arrieta *et al.*, 2014; Pereira de Abreu *et al.*, 2012; Pereira de Abreu *et al.*, 2010)

Polymer flat film / sheet production process is shown in *Figure 2.13.*

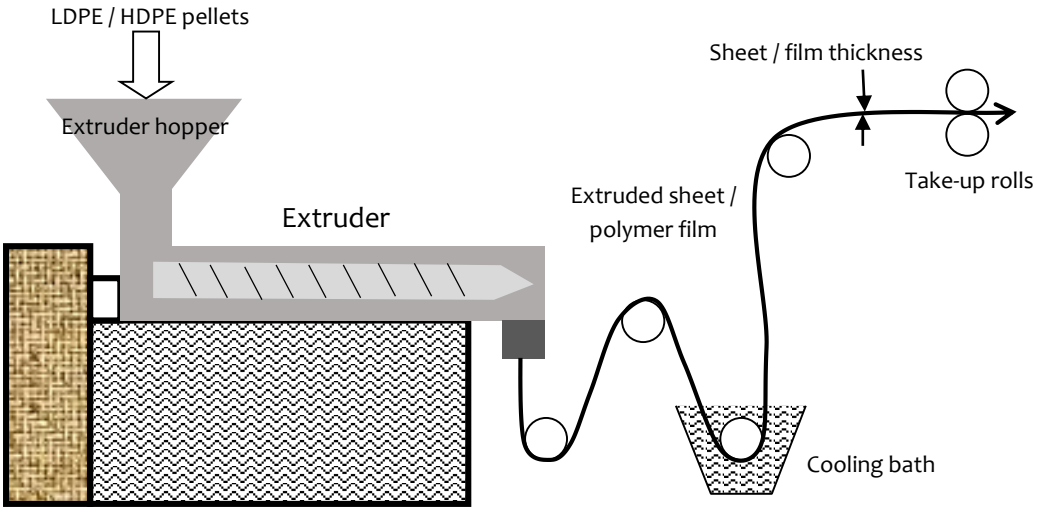


Figure 2.13: *Polymer flat film / sheet production process*

2.19 Migration in Polymeric Packaging Materials

In the food package terminology, migration is the transfer or release of substances initially present in the package into the food. Substances that are transferred to the food as a result of contact or interaction between the food and the packaging material are often referred to as migrants. Plastics contain low MW (molecular weight) substances including monomers and oligomers and various additives such as plasticizers that can migrate. Arvanitoyanis & Bosnea, (2004), illustrated how substances diffuse through the material until they reach the inside surface of the package from where they are partially transferred to the headspace or dissolved in food. This may result in loss of food quality, due to flavor or colour changes or it may make the food toxic without perceptibly altering its organoleptic properties. So European Union (EU) legislation imposes specific migration limits upon an individual substance with the potential to migrate from plastics to foodstuffs according to their individual toxicity (Dopico-Garcia & Gonzalez-Rdriguez, 2003). According to the review done by Castilo & Borrell, (2018) the EU legislation for overall migration limit (OML) is 10mg of substance per dm^2 of the food. However, it is important to note that migration is a two way

process because constituents of the food can also migrate into the food packaging materials. An example is the 'scalping' of flavor compounds from fruit juice by plastics. On the other hand, compounds present in the environment surrounding the packaged food can be sorbed by the packaging and migrate into the food. For example, perfumes from soaps can be picked up by fatty foods under certain circumstances like nature of packaging material and proximity of the two products at the time of exposure (Arvanitoyanis & Bosnea, 2004).

A lot of work has been done on the extent of migration of many food contact polymeric packaging materials which contain residues of the polymerization process or additives employed to facilitate processing. Migration of substances is a function of time, temperature, volatility of the migrant and type of media (aqueous, acidic or basic media) the migrant is in. In the work carried out by Till *et al*, (1982) in majority tests, migration was approximately proportional to the square root of time; it also increased significantly with a rise in temperature, and proportional to the initial concentration of migrant in the polymer. It was also reported that the physical steps involved in the migration included, the diffusion of migrant from the interior of the film to the surface, where it can dissolve in the external of the food phase.

In the same study carried out by Till *et al*, (1982) on the migration of dibutylhydroxytoluene (BHT) from HDPE to foods (skim and whole milk, margarine and mayonnaise) and food simulants found no accumulation of BHT in the aqueous phase. However they reported that when corn oil was used as the stimulant, all the BHT migrated during the 50day test period. Migration observed at 4^{o}C for skim and whole milk was greater than that for water. It was felt that some ingredients from milk could have penetrated the HDPE, even at such a low temperature, and modify the migration propensity of the BHT, while in a study by Miltz *et al*, (1988) investigated loss of the antioxidants BHT from HDPE film. The volatilization of the antioxidant from the polymer surface was reported to be

the controlling parameter for mass transfer rather than diffusion of the antioxidant through the bulk polymer to the surface layer. Oatmeal cereal packaged in HDPE impregnated with high level of BHT had an extended shelf-life compared to HDPE impregnated with low level of BHT, as a result of adsorption by the cereal of antioxidant from the package. study carried López-de-Dicastillo *et al*., (2012) reported that the release of ascorbic and ferulic acid was higher in aqueous simulants while the release of quecitin and green tea extract was higher in ethanolic simulants. In a study by Wessling *et al*, (2000) compared the loss of α-tocopherol and BHT from LDPE film packed with oatmeal. Result showed that BHT was rapidly lost at all temperatures, where as, α-tocopherol was retained for a longer period of time. The oatmeal had undergone the least change during the 8-10 week storage period.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

The underlisted mterials and equipment were used for this research.

a. Ehuru spice Michael Okpara University of Agriculture Umudike Abia State.
b. Polysulfone Polymer resin procuredfrom Sigma Aldrich South Africa
c. Fresh peanuts purchased from Ekeonuwa market Owerri.
d. Alpha tocopherol, Tetrahydrofuran (THF), *N*-methylpyrrolidinone (NMP), acetic acid, chloroform, sodium thiosulphate, potassium iodide (KI), starch indicator, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH). (All these chemicals were procured from Sigma Aldrich, South Africa)
e. Gas Chromatography GC-MS; Shimadsu (Japan)
f. Universal tensile tester; TA. XT (Stable Microsystems SMD, England) scanning electron microscope (SEM) for morphological analysis; AEM-3200 scanning electron microscope (KYKY, China)
g. Fourier Transform Infrared Spectroscopy (FT-IR); (Agilent cary 630 spectrophotometer, US).
h. Casting blade
i. Oxygen permeability tester: QT-OPT 300
j. DSC equipment for thermal analysis; A Perkin- Elmer differential scanning calorimeter, Japan.
k. CDR LAB line Analyser; 809x 252 CDR food lab
l. UV-VIS Spectrophotometer; Shimadzu (Japan)
m. Oven: (280 litre/clad/ HYD)
n. Glass plate
o. Coagulation bath.
p. Heat sealer (Thermal impulse sealer, mode PE5- 260)
q. Rotary evaporator (13284 Heidolph rrotary everporator, USA)

r. Blender, (Kenwood BL380, UK)
s. Magnetic stirrer.

3.2 Methods

3.2.1 Preparation of the antioxidant spice extract

The fresh ehuru collected from Michael Okpara University of Agriculture Umudike Abia State, was roasted for easy decortication to prepare the spice extract the method used by Womeni *et al*., (2013) was used. Decorticated ehuru spice was sorted to remove debris,extraneuos/ foreign particles and infested grain. It was dried in an oven at a temperature of 50^{o}C to constant weight. The dried ehuru was milled to very fine particle size using a laboratory blender and sieved by passing it through a sieve size of 0.5mm.

200g of the spice wereadded into 500ml ethanol(70% v/v), stired for 48hours at room temperature. The mixture was filtered and residue was further treated with 300ml ethanol to ensure complete exraction of the phenolic compounds.The filtrate wasintroduced into rotary evaporator at 65^{o}C for the removal of solvent. The extract was further dried in an oven to obtain a gelly like substance which was stored at 4^{o}C prior further analysis.

3.2.2 Production of Test peanut oil Sample

The cleaned peanut was roasted, milled, and pressed to release the oil using mechanical press. The exracted oil was heated to remove any inherent water molecule.

3.2.3 Preparation of Active packaging material

Three types of films were produced. One containing different percentage compositions of only the Ehuru Antioxidant Extract (EAE), another containing a-tocopherol (AT) and the last containing a blend of equal percentage of a-tocopherol and (EAE). EAE was added to Polysulfone (PSF) resin at different percentage compositions of 0, 1, 2.5, 5% w/w spice. In another Polysulfone resin

was incorporated 5% of a standard natural antioxidant, alpha tocopherol (AT) and also an equal blend of 5% EAE/ AT

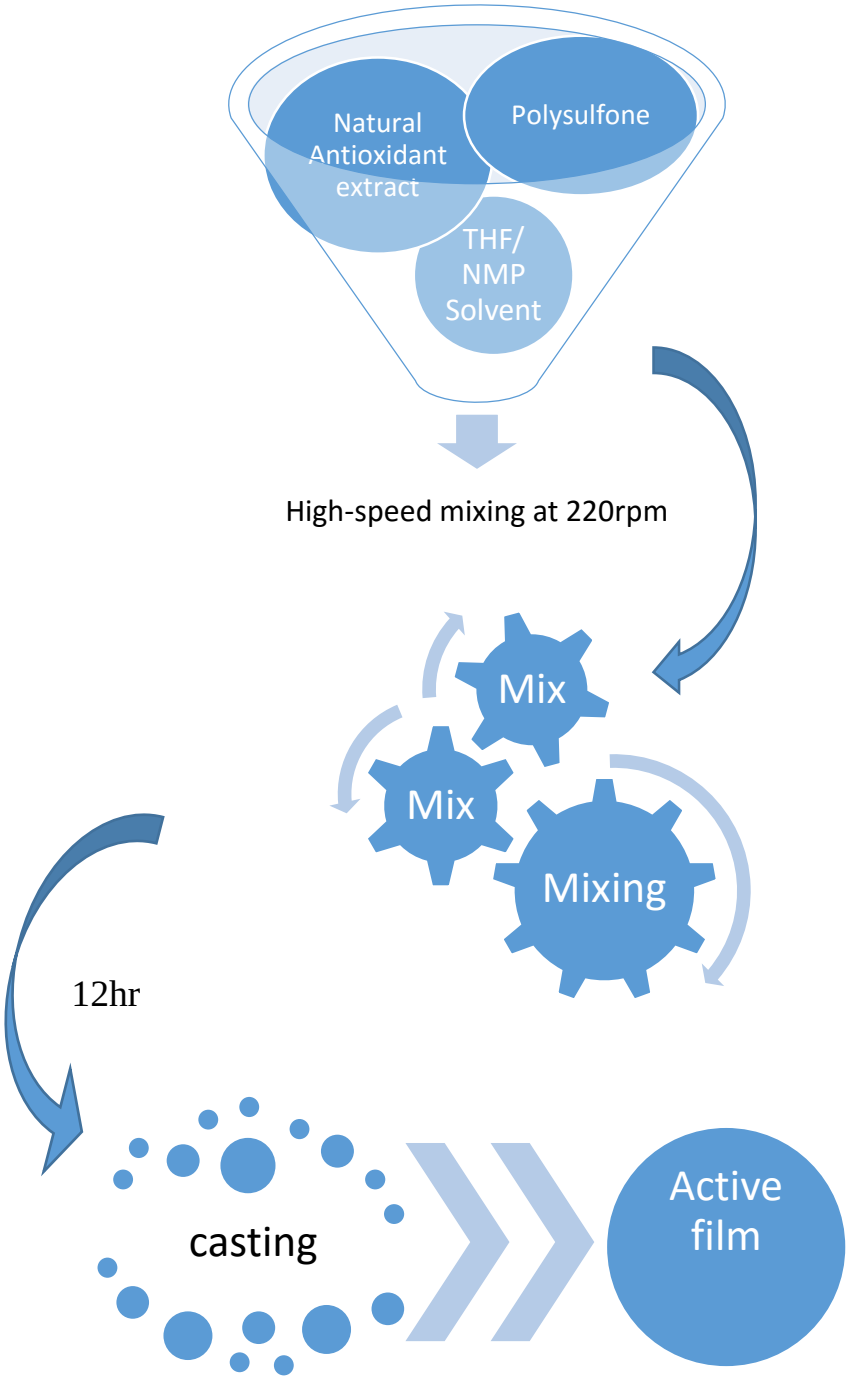


Figure 3. 1: *Process flow diagram for active polymer film preparation*

Tetrahydrofuran (THF) and *N*-methylpyrrolidinone (NMP) were used as solution solvent, in ratio 3:1. 10 g of PSf was dissolved in the NMP/THF mixture at room temperature, the mixture was agitated under a magnetic stirrer set at high speed (220rpm) for 24h until complete dissolution. The resulting solution was mixed with the varying compositions of EAE and a-tocopherol (AT), PS (control) . The films were produced by casting on a glass plate using a casting blade. The casting solution was kept at room temperature for at least 2 hours before casting in order to remove air bubbles. The newly cast was immediately immersed in distilled water coagulation bath within 10s to form the active film which was peeled off the glass plate. After immersion, the residue solvent in the film were removed by immersing in distilled water for at least 24 hours.

3.2.4 Packaging of Peanut Test oil Samples in the Active films

The active films of EAE, AT, and EAE/AT produced blended and used to package fresh peanut oil samples and stored over time.

3.2.5 Chemical analysis of the Extract

3.2.5.1 GCMS Analysis of the Ehuru Extract

The ethanol extract component identification was achieved by the GC-MS analysis using Shimadsu (Japan) Gas Chromatography GC-MS unit equiped with fused silica column RXi-5MX with column dimention of 0.25um thickness and 0.25mm diameter and 30.0mm length. Ultra- high purity helium (99.8%) was used as carrier gas at a constant flow rate of 1.0mL/min. The injection, transfer line and ion source temperatures were all 290˚C. The ionizing energy was 70eV. Electron multiplier voltage was obtained from auto-tune. The oven temperature was programmed from 60˚C (hold for 2mins) to 280˚C at a rate of 3˚C/min. The extract were diluted with ethanol (1/100, v/v) and an injection volume of 1 µl was employed, with a split ratio 30: 1.

The percentage composition of the crude extract constituents was expressed as a percentage by the peak area. Total GC running time was 36 min.The identification and characterization of chemical compounds in various crude extracts were based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum National Institute of Standards and Technology (NIST) library search

3.2.6 Active package analyses.

3.2.6.1Tensile Mechanical Properties

The tensile strenth (TS), percentage tensile elongation at break (E) of the active film were determined at room temperature using a tensile testing machine; TA. XT2 (Stable Microsystems SMD, England) according to ASTM standard method D882. The TA.XT was equipped with 7.5kg static load cell.

Sample Films were cut into rectangular shape (10x 2.5 cm) and mounted between the grips of the TA- XT instrument. The initial grip spacing and cross head space were set at 30mm and 25mm/min respectively. The tensile strength was expressed as the maximum force at break divided by the initial cross-sectional area of the sample film as shown in equation 3.1

$$TS = F_{max}/A \qquad 3.1$$

while the elongation at break (EAB) is the percentage of the original length at break as shown in equation 3.2

$$EAB\ (\%) = L/L_o \times 100 \qquad 3.2$$

TS and the (EAB) were expressed in MPa and percentage (%) respectively. Values are the mean of replicated 3 measurements for each film sample (Doshi *et al.*, 2015) and the thickness of the films were measured using micrometer screw gauge (MDC-25S Japan).

3.2.6.2 FTIR Characterisation of the active film

The active films were characterized by Fourier transform infrared spectrophotometer(FTIR) (Agilent cary 630 spectrophotometer, US). This was done in other to abserve the structural interactions between the polysulfone and the EAE or α- tocopherol. Films were conditioned at room temperature for 7days in a desiccator containing silica gel. This was done to have dehydrated films. 0.6-0.8 mg of film samples were placed on the crystal cell and the cell (sensor surface) was clamped onto the mount of FTIR spectrophotometer.The scan wave number was from 4000 cm^{-1} to 650 cm^{-1}. 16 scans per spectrum were recorded with a resolution of 4 cm^{-1}. The spectra obtained were used to determine possible interactions of functional groups between the polysulfone molecules and the EAE or a- tocpherol (AT).

3.2.6.3Thermal Properties; DSC ANALYSIS:

A Perkin- Elmer differential scanning calorimeter was used to estimate the thermal transitions of the new active films.5 – 8mg of the PSF active polymer films was measured. Using a Differential Scanning Calorimeter (DSC) at the heating rate of 20°C/min from 0 to 340°C. The glass transition temperature (T_g), and melting temperature (T_m) of the active polymer films were determined from the DSC curves (Byun, Kim & Whiteside, 2010)

3.2.6.4 Barrier Properties (Oxygen Transmission Rate (OTR)/ Permeability Properties)

OTR of the films of average thickness cm were determined according to ASTM D3985 at 65±5% relative humidity and tested at 25°C, using oxygen permeability tester: QT-OPT 300. The test samples were however conditioned at the testing cell for 10 hours. Oxygen permeability was calculated by multiplying the oxygen transmission rate by the film thickness. Tests were carried out in triplicate and mean values obtained.

3.2.6.5 Morphological analysis;

The surface and cross section of the films were analyzed by using the Scanning Electron Microscopy (SEM**).** To observe the films microstructure and level of antioxidant, extract dispersion in the polymer matrix. AEM-3200 scanning electron microscope (KYKY, China) was used for the study. The films were mounted on the specimen holder with aluminium tape and then coated with gold for 30 s at 20 mA. All samples were examined using an accelerating beam at a voltage of 25.0 kV. Magnification was at 5,000 x.

3.2.6.6 Radical scavenging (antioxidant) activity of the Active film

Oxidation protection effect of the active film was determined using the method of Shojaee-Aliabadi *et al*., (2013) as described by Jouki, *et al.*, (2014). 0.1g of the active polymer films was cut into small pieces and mixed with 2ml of methanol. The mixture was vigorously vortexed for 3 min and allowed to stand at room temperature for 3h and centrifuged at 2300 rpm for 10 min. The supernatant obtained was analyzed for DPPH radical scavenging activity. An aliquot of methanol extract (1ml) was mixed with 2ml of 0.1mM DPPH in methanol. The mixture was vigorously vortexed for 1 min and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517nm using a UV- VIS spectrometer. The methanol was used as control and was mixed with 0.1mM DPPH. DPPH radical scavenging activity was calculated using equation 3.3

Radical scavenging activity % = (($A_{reference}$ - A_{sample})/$A_{reference}$)x100 3.3

where A_{sample} is the absorbance of sample and $A_{reference}$ is the absorbance of the DPPH solution without the sample film (Singh & Ragini, 2004).

3.2.7Active package test on lipid food.

3.2.7.1 Determination **of oxidation rate of packaged peanut oil;**

The oxidation of processed peanut oil packaged in the produced active films was monitored for accumulation of peroxides. The package 5cm x5cm was prepared using a heat sealer (Thermal impulse sealer, mode PE5- 260) filled with $10cm^2$ peanut oil, heat sealed and stored in the dark at room temperature for 9 weeks. 4 packages (sachets) where prepared one from the control film and the other 3 sachetes each from one of theactive films (EAE,AT,EAE/AT). All were labelled for identification. The oxidation processs was monitored by taking samples for 9 weeks. The peroxide (PV) and anisidine (AV) valueswhere determined.

3.2.7.1.1 Peroxide value (PV)

The peroxide value (meq O_2/kg oil sample) was determined according to the Official AOAC 965.33 (1969) iodometric method with some modification. 2.5 g of the samples were weighed into 250 ml conical flasks and 30 ml of 3:2 (v/v) acetic acid/chloroform mixture were added to the samples and the flasks were swirled to dissolve the samples. Aliquots of 0.5 ml of 2% KI solution were added to the samples and the reaction mixtures were left to stand for 1 min at room temperature (about 28^OC) with occasional shaking. 30 mls of distilled water were then added to the sample and the resulting reaction mixtures were each titrated with 0.1 N standardized sodium thiosulphate to a pale yellow colour, then 2 ml of starch indicator were added and the titration was continued to a colourless end-point. A blank titration was carried out using a sample containing all the above reagents except oil sample. Triplicate measurements of peroxide values of each treatment concentration were carried out initially at weekly intervals up to the 9^{th} week The PV was calculated using equation 3.4

$$PV = (S - B) \times N/M \quad 3.4$$

Where, S and B are the volumes of titrant for the sample and blank, respectively, and N is the normality of sodium thiosulphate solution. M is mass of sample in grams.

3.2.7.1.2 P - anisidine value (AV)

Anisidine value measures the amount of carbonyl compounds, aldehydes (principally 2-alkanals and 2,4 dienals) in the oil sample. P-AV was determined using the CDR food lab line analyser equipment (809 x 252 CDR food lab) shown in fig. 3.2. Its working principles followed AOCS standard method CD 18 – 90. 2 mls of p- anisidine reagent in a cuvette was incubated for 10 min in the incubation cell. 'P- anisidine analysis' was selected. Blank reading of the reagent was taken by inserting the cuvette containing the reagent in the reading cell. 10um of the oil sample was introduced into the cuvette containing the blank and shaken to ensure thorough mixture. The p-anisidine value was read by inserting the cuvette containing the mixture in the reading cell.The aldehydes derived from the secondary oxidation of the fat matrix reacts with the p-anisidine determining the variation in the absorbance was measured at 366nm. This test has an enhanced sensitivity for unsaturated aldehydes, especially 2, 4-dienals, but does not measure the ketonic secondary products of oxidation. The lower the p- anisidine value the better the oil analysed.

Fig 3.2: CDR food lab line analyser

3.2.8 Experimental design

A 3x3x1 factorial design comprising of 3 antioxidant active agent, three concentrations and 1 polymer resin given 9 samples used for the package analysis. 4 test analysis, each given a total of 36 samples. A 4x2x9 factorial design comprising of 4 package, 2 test analysis and 9interval of time giving rise to 72samples was used for the oxidative analysis test. For the EAE active package analysis, a 4x2x9 factorial design comprising of 4 conentrations 2 test analysis and 9 interval of time (72 samples) was also used for oxidative rancidity test. Therefore, a total of 150 samples were used**.** Performance data were presented as tables, graphs and charts where necessary.

3.2.9 Statistical Analysis

The data obtained from different analyses were subjected to various statistical analyses which include simple descriptive mean and standard deviation, One-way analyses of variance was carried out. The SPSS computer program (SPSS Inc., Chicago, IL, USA) was used. Differences in pairs of mean values were evaluated by Duncan multiple range test (SAS) for a confidence interval of 95%.

CHAPTER FOUR

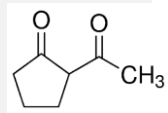
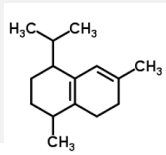
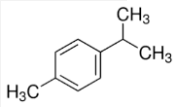
RESULTS AND DISCUSSION

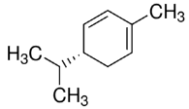
4.1 RESULTS

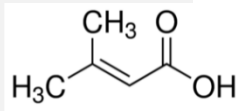
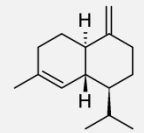
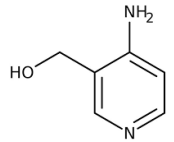
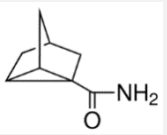
Gas Chromatography (GC-MS) analysis of the phenolic components of Ehuru Extract

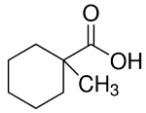
Table 4.1 presents the major phenolic constutuents identified in the ethanolic extract of the Ehuru spice, their chemical structure and molecular weihgt.

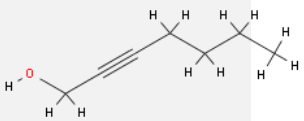
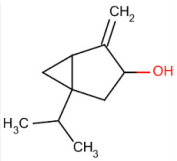
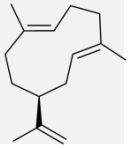
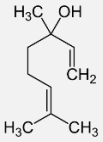
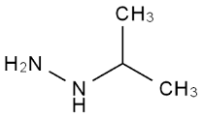
Table 4.1 Major Phenolic compounds identified in ethanolic extract of Ehuru

S/N	**Compound**	**Retention Time (min)**	% Composition	**MW (g/mol)**	**Molecular formular**	**Structure**
1	2-Acetylcyclopentanone	19.094	9.83	126.155	$C_7H_{10}O_2$	
2	Isopropyl-4,7-dimethyl-1-hexahydronaphthalene	24.530	8.34	204.3511	$C_{15}H_{24}$	
3	p-cymene	9.750	7.43	134.21	$C_{10}H_{14}$	

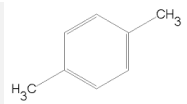
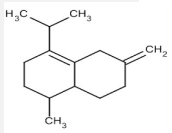
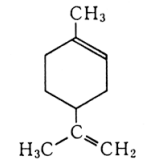
S/N	Compound	Retention Time (min)	**% Composition**	MW (g/mol)	Molecular formular	Structure
4	Alpha-phellandrene	9.104	7.12	136.238	$C_{10}H_{16}$	

5	2-Butenoic acid	21.354	6.35	86.09	$C_4H_6O_2$	
6	Gamma-muurolene	24.341	6.20	204.357	$C_{15}H_{24}$	
7	3-pyridinemethanol	30.063	5.95	109.13	C_6H_7NO	
8	Tricyclo[2.2.1.0(2,6)]heptane	21.732	5.91	94.154	C_7H_{12}	

S/N	Compound	Retention Time (min)	**% Composition**	MW (g/mol)	Molecular formular	Structure
9	4- penthyl clohexyi -ester	21.961	3.19	252.398	$C_9H_{14}O_2$	

10	2-Octyn-1-ol	19.449	2.94	126.199	$C_8H_{14}O$	
11	Bicyclo(3.1.0)hexan-3-ol	15.501	2.50	98.145	$C_{10}H_{18}$	
12	Germacrene D	27.666	2.42	204.357	$C_{15}H_{24}$	
13	Linalool	12.205	2.33	154.253	$C_{10}H_{18}O$	
14	1-Methyl-3,3-pentamethylenediaziridine	16.914	2.30			

S/N	Compound	Retention Time (min)	**% Composition**	MW (g/mol)	Molecular formular	Structure

15	P-Xylene	5.361	2.29	106.168	C_8H_{10}	
16	4,7-Methanoazulene	23.935	2.10	140.185	$C_{11}H_8$	
17	D-Limonene	9.865	1.18	136.24	$C_{10}H_{16}$	

4.1.1 Physical properties of the active packaging film

The results obtained for the mechanical (tensile), antioxidant and barrier property of the active film are shown in Table 4.2. Shown in Table 4.3 are the results obtained for the barrier properties of the active packaging film. Table 4.4 shows the results obtained for the thermal properties of the active packaging films.

Table 4.2: Mechanical and Antioxidatant Properties of the Active Films.

Property	0.0%EAE	1.0%EAE	2.5%EAE	5.0%EAE	5.0%AT	5.0% EAE/AT	LSD
Tensile stress (MPa)	8.132^{a}**±0.31**	7.739^{b}**±0.71**	7.538^{c}**±0.25**	7.426^{d}**±0.34**	5.636^{f}**±0.22**	6.405^{e}**±0.54**	0.022
Elongation at Break (%)	2.002^{d}**±0.72**	2.353^{c}**±0.11**	2.673^{b}**±0.33**	3.221^{a}**±0.09**	1.702^{f}**±0.55**	1.751^{e}**±0.23**	0.0074
Antioxidative Property (%)	3.100^{f}**±0.10**	10.077^{e}**±0.47**	22.590^{d}**±0.24**	48.097^{b}**±0.70**	51.587^{a}**±0.26**	40.961^{c}**±0.67**	0.858

Values are the means of duplicate determinations

a,b.... means with the same superscript inthe same column are not significantly different at p> 0.05

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

Table 4.3: Barrier Properties of the Active Films.

Film description	L (cm)	P (cmHg)	flow rate dV/dt (cm^3/s)	Permeability of film [$mL(STP)cmcm^{-2}S^{-1}(cmHg)^{-1}$]
0.0%EAE	0.006	90.0077	0.1256^{b}	$8.721^{c}x10^{-7}$
1.0%EAE	0.014	90.0077	0.0463^{e}	$7.502^{d}x10^{-7}$
2.5%EAE	0.005	90.0077	0.0593^{d}	$3.431^{e}x10^{-7}$
5.0%EAE	0.007	90.0077	0.0277^{f}	$2.244^{f}x10^{-7}$
5.0% EAE/AT	0.017	90.0077	0.1079^{c}	$2.1228^{b}x10^{-6}$
5.0%AT	0.014	90.0077	0.2209^{a}	$3.5791^{a}x10^{-6}$
LSD	**0.0058**	**NS**	**0.0022**	**0.0066**

Values are the means of duplicate determinations

a,b.... means with the same superscript inthe same column are not significantly different at p> 0.05

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

Table 4.4: DSC Thermal Properties of the Active Films

Samples	1st Onset temp T_o (°C)	T_g (°C)	T_c (°C)	T_m(°C)	Degradation Temp (°C)
CON	62.09[b]	87.5[b]	192.6[a]	230.1[c]	255[d]
EAE	62.01[b]	87[b]	153[d]	210[d]	284.5[a]
AT	63.85[a]	87.08[b]	167[c]	247[a]	279.5[b]
EAE/ AT	62.01[b]	102.05[a]	184.5[b]	237[b]	257.6[c]
LSD	0.71	0.82	2.04	2.45	1.43

Values are the means of duplicate determinations

a,b.... means with the same superscript in the same column are not significantly different at p> 0.05

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

4.1.2 FTIR Characterisation of the active film

The result of the FTIR analysis for 0% ehuru polymer composite (control),5% ehuru polymer composite , 5% AT polymer composite and 5% AT/ehuru polymer composite are shown in Figs 4.1, 4.2, 4.3 and 4.4 respectively.

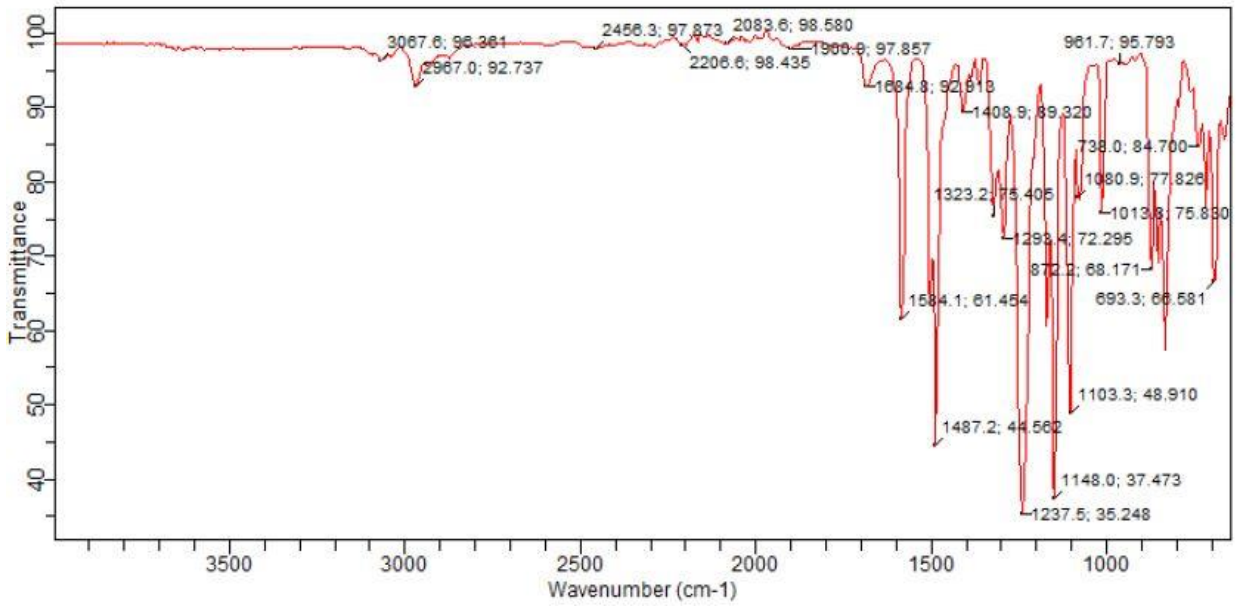


Fig. 4.1: FTIR v*ibrational bands* for 0% EAE/PSF Polymer compositions

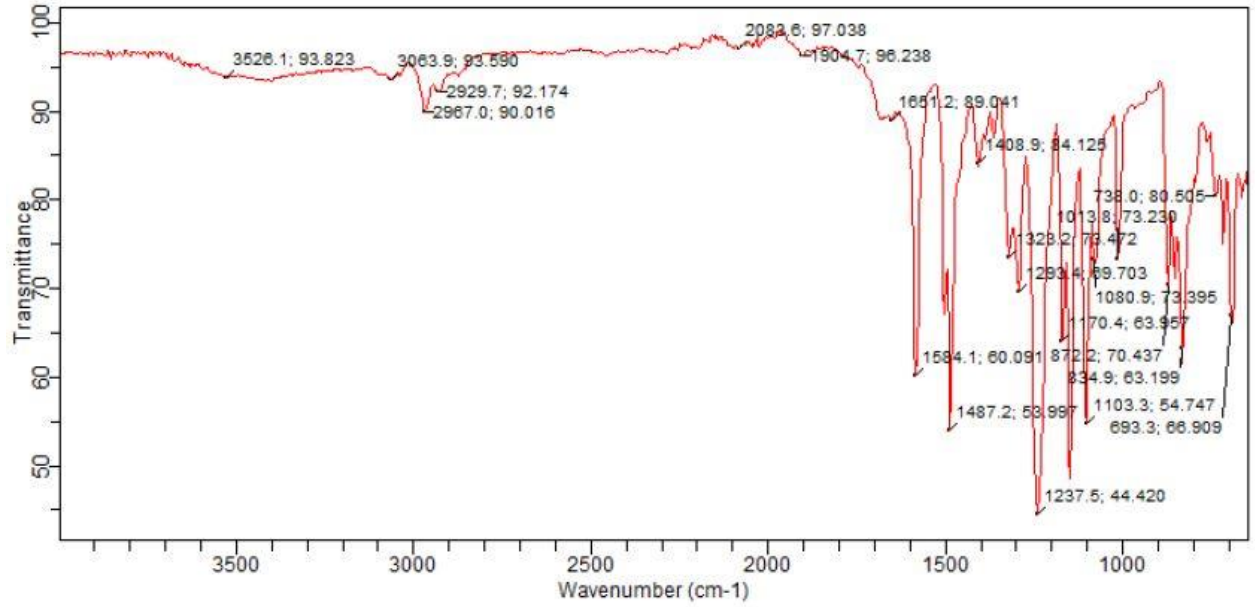


Fig. 4.2:*FTIR* v*ibrational bands* for 5% EAE / Polymer compositions

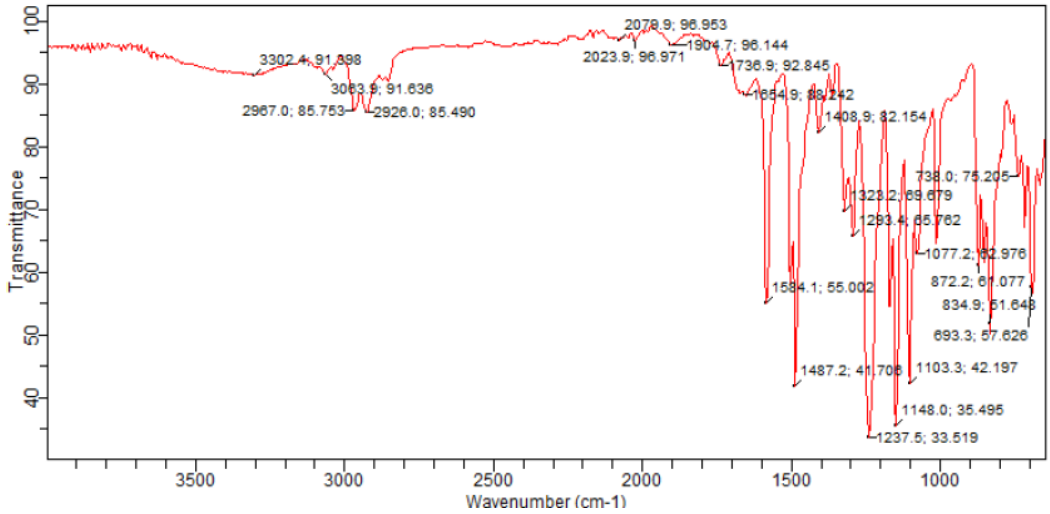


fig 4.3 FTIR v*ibrational bands* for 0.5% α –T / Polymer compositions

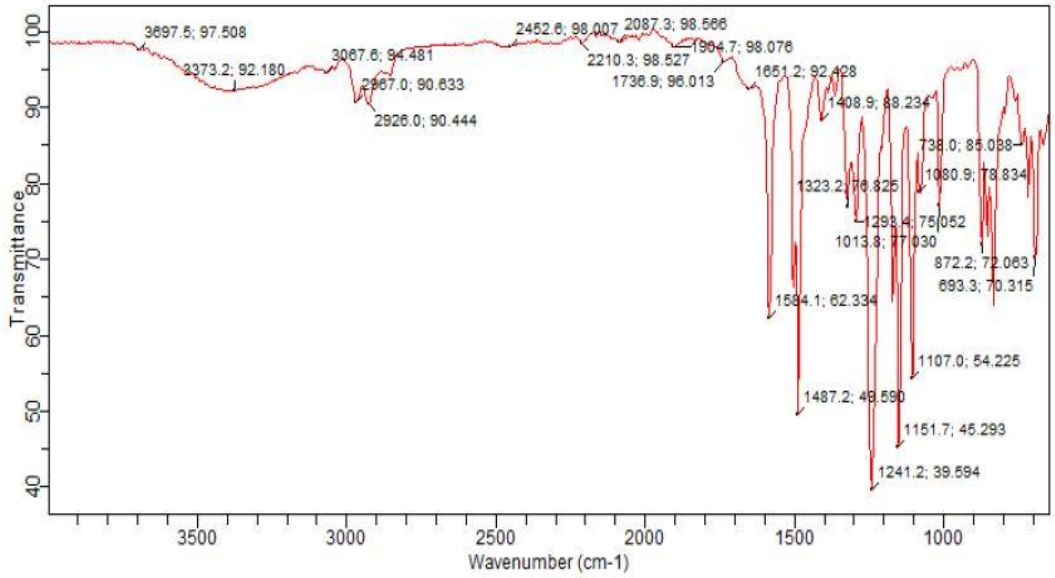
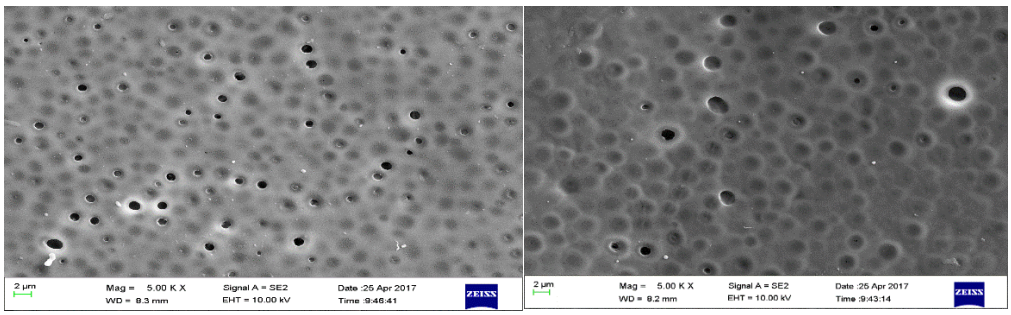


Fig. 4.4 :*FTIR vibrational bands* for 2.5% EAE / 2.5% α –T / Polymer compositions

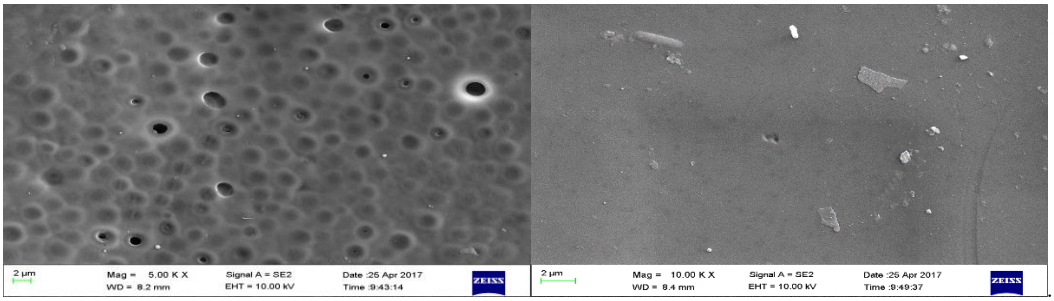
4.1.3Morphological analysis;

The results of the SEM analyses are shown in fig 4.5 A for 0%EAE, B:1% EAE, C:2.5% EAE, D:5%EAE, E: 5%blend EAE/AT and F:5%AT



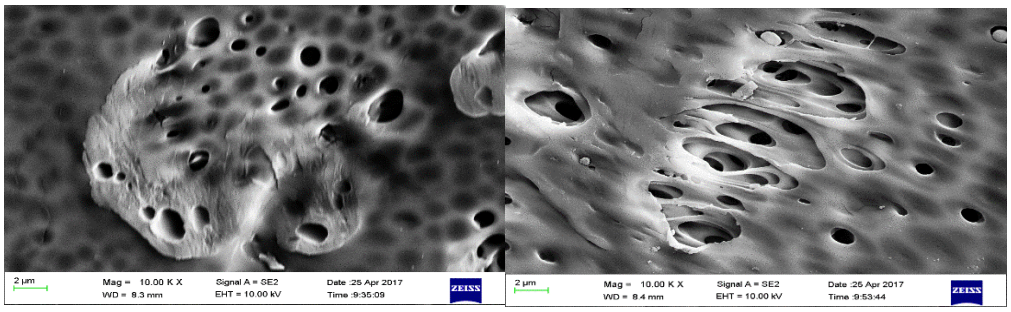
A: control; 0% EAE

B: 1% EAE



C: 2.5% EAE

D: 5% EAE



E: 5% blend of EAE/AT

F: 5% AT

Fig 4.5: SEM Micrograph of the antioxidative Active PSF films

4.1.4 OXIDATION ANALYSIS (RANCIDITY) OF THE OIL PACKAGED WITH ACTIVE FILM

The results of oxidation analysis (rancidity) of peanut oil packaged and stored with the active film are shown in Tables 4.5 –4.12. Table 4.5 shows the result of peroxide value (PV) of stored penut oil packaged with film containing different antioxidants. Table 4.6 shows the result of p-Anisidine of stored penut oil packaged with film containing different antioxidants. Table 4.7 shows the result of peroxide value (PV) of stored penut oil packaged with film containing different concentrations of EAE. Table 4.8 shows the result of p- Anisidine value of stored penut oil packaged with film containing different concentrations of EAE. The result of the effect of various antioxidants on the PV of the actively packaged peanut oil is shown in Table 4.9, while the effect of different antioxidant on the p-Anisidne value of the actively packaged peanut oil is shown in Table 4.10. Table 4.11 shows the effect of different concentrations on the P- Anisidine value of the actively packaged peanut oil while Table 4.12 shows the effect of different concentrations on the PV of the actively packaged peanut oil.

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Table 4.5: Peroxide value (PV) of stored Peanut oil packaged with film containing different Antioxidants

Duration of storage (Weeks)	PV (meq/kg of oil)			
	Control	EAE	EAE/AT	AT
0	4.5±0.00^{j}	4.5±0.00^{j}	4.5±0.00^{i}	4.5±0.00^{j}
1	5.61±0.15^{i}	5.26±0.05^{i}	5.44±0.42^{i}	5.50±0.32^{i}
2	7.43±0.12^{h}	6.34±0.09^{fg}	6.62±0.34^{i}	5.33±0.41^{h}
3	8.32±0.22^{g}	6.41±0.28^{f}	7.31±0.44^{f}	6.85±0.05^{g}
4	9.60±0.25^{f}	6.63±0.44^{f}	8.63±0.26^{e}	8.70±0.33^{f}
5	13.44±0.17^{e}	8.25±0.45^{e}	7.05±0.55^{g}	12.83±0.84^{b}
6	18.22±0.35^{b}	9.50±0.65^{b}	12.21±0.75^{c}	11.24±0.52^{d}
7	18.00±1.52^{b}	9.11±0.51^{c}	12.70±0.63^{a}	10.50±0.44^{e}
8	17.25±1.33^{d}	8.85±0.32^{d}	11.25±1.02^{d}	12.75±0.70^{c}
9	20.50±1.10^{a}	9.82±1.50^{a}	12.30±1.35^{b}	14.20±0.65^{a}
LSD	**0.32**	**0.03**	**0.06**	**0.08**

Values are the means of duplicate determinations

a,b.... means with the same superscript in the same column are not significantly different (p> 0.05)

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

Table 4.6: P- Ansidine value of stored Peanut oil packaged with film containing different Antioxidants

Duration of storage (Weeks)	P- Anisidine value			
	CON	**EAE**	**EAE/AT**	**AT**
0	$9.5^{j} \pm 0.00^{j}$	9.5 ± 0.00^{j}	$9.5^{j} \pm 0.00^{j}$	$9.5^{j} \pm 0.00^{j}$
1	12.01 ± 0.00^{i}	10.44 ± 0.05^{h}	14.46 ± 0.16^{i}	14.84 ± 0.32^{i}
2	14.22 ± 0.00^{h}	11.62 ± 0.03^{g}	17.32 ± 0.20^{h}	16.22 ± 0.44^{h}
3	18.32 ± 0.17^{g}	13.31 ± 0.10^{f}	19.11 ± 0.78^{g}	18.33 ± 0.21^{g}
4	23.45 ± 0.25^{f}	14.63 ± 0.41^{f}	21.33 ± 0.43^{f}	20.5 ± 0.38^{f}
5	32.25 ± 0.44^{e}	16.05 ± 0.32^{e}	22.5 ± 0.15^{e}	21.05 ± 0.02^{e}
6	35.27 ± 0.04^{d}	24.21 ± 0.04^{d}	24.33 ± 0.05^{d}	28.2 ± 0.34^{d}
7	38.75 ± 0.05^{c}	24.25 ± 0.56^{d}	24.83 ± 0.25^{c}	28.45 ± 0.78^{c}
8	43.25 ± 0.30^{b}	26.75 ± 0.05^{c}	30.52 ± 0.07^{b}	34.75 ± 0.59^{b}
9	49.37 ± 0.24^{a}	30.45 ± 0.15^{b}	35.50 ± 0.20^{a}	40.58 ± 0.60^{a}
LSD	**0.14**	**1.25**	**0.28**	**0.28**

Values are the means of duplicate determinations

a,b.... means with the same superscript inthe same column are not significantly different ($p > 0.05$)

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

Table 4.7: PV (meq O_2/ kg oil) of Peanut oil packaged with film containing different concentrations of EAE

Duration of storage (Weeks)	PV of samples (meq/kg of oil)			
	0%**EAE**	1%**EAE**	**2.5% EAE**	**5% EAE**
0	4.5±0.00^{j}	4.5 ±0.00^{j}	4.5±0.00^{j}	4.5±0.00^{j}
1	5.61±0.15^{i}	5.52±0.20^{i}	5.35±0.00^{i}	5.26±0.05^{i}
2	7.43±0.12^{h}	6.57±0.20^{h}	6.43±0.05^{g}	6.34±0.09^{fg}
3	8.32±0.22^{g}	7.86±0.30^{g}	6.52±0.30^{g}	6.41±0.28^{f}
4	9.60±0.25^{f}	8.38±0.34^{f}	7.05±0.07^{f}	6.63±0.44^{f}
5	13.44±0.17^{e}	11.21±0.20^{e}	9.22±0.05^{d}	8.25±0.45^{e}
6	18.22±0.35^{b}	15.1±0.30^{a}	10.45±0.02^{a}	9.50±0.65^{b}
7	18.00±1.52^{b}	13.83±0.10^{b}	10.12±0.10^{b}	9.11±0.51^{c}
8	17.25±1.33^{d}	12.40±0.06^{d}	9.84±0.15^{c}	8.85±0.32^{d}
9	20.50±1.10^{a}	13.02±0.00^{c}	9.01±0.12^{d}	9.82±1.50^{a}
LSD	**0.24**	**0.03**	**0.21**	**0.03**

Values are the means of duplicate determinations

a,b.... means with the same superscript inthe same column are not significantly different (p> 0.05)

CON = Control, EAE = ehuru antioxidant extract.

Table 4.8: p-Anisidine value of Peanut oil packaged with film containing different concentrations of EAE

Duration of storage (Weeks)	PV of samples (meq/kg of oil)			
	0%**EAE**	1%**EAE**	**2.5% EAE**	**5% EAE**
0	9.5±0.00^{j}	9.5±0.00^{j}	9.5±0.00^{i}	9.5±0.00^{j}
1	12.01±0.20^{i}	14.05±0.04^{i}	12.15±0.32^{i}	11.44±010^{i}
2	14.22±0.12^{h}	14.28±0.10^{h}	13.38±0.00^{h}	12.2±0.50^{h}
3	18.32±0.25^{g}	16.85±0.35^{g}	15.34±0.45^{g}	13.31 ±0.44^{g}
4	23.45±0.22^{f}	19.54±0.52^{f}	14.29±0.04^{f}	14.63±0.15^{f}
5	32.25±0.17^{e}	22.05±0.20^{e}	17.64±0.25^{e}	15.75 ±0.10^{e}
6	35.27±0.35^{d}	23.44±0.02^{d}	21.23±0.01^{d}	16.25±0.05^{d}
7	38.75 ±1.52^{c}	26.15±0.00^{c}	26.81±0.33^{c}	18.25±0.35^{c}
8	43.25±1.33^{b}	34.71±0.30^{b}	28.52±0.15^{b}	26.75 ±0.50^{b}
9	49.37±1.10^{a}	35.50±0.20^{a}	29.50±0.22^{a}	28.5±0.10^{a}
LSD	**0.24**	**0.31**	**0.2**	**0.2**

Values are the means of duplicate determinations

a,b.... means with the same superscript inthe same column are not significantly different at p> 0.05

CON = Control, EAE = ehuru antioxidant extract.

Table 4.9: Effect of antioxidant on the PV of actively packaged peanut oil.

SAMPLES	PV(meq O_2/ kg oil) of peanut oil
CONTROL	12.29 ± 0.90^{a}
EAE	7.35 ± 2.00^{c}
EAE/AT	8.8 ± 1.07^{b}
AT	9.24 ± 3.55^{b}
LSD	**1.78**

Values are the means of duplicate determinations

a,b.... means with the same superscript in he same column are not significantly different ($p > 0.05$)

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

Table 4.10 Effect of antioxidants on the P anisidine value of actively packaged peanut oil.

SAMPLES	P anisidine value of peanut oil
CON	27.64± 1.05[a]
EAE	18.12± 1.57[bc]
EAE/AT	21.94± 1.54[b]
AT	23.24± 1.06[b]
LSD	**3.23**

Values are the means of duplicate determinations

a,b.... means with the same superscript in the same column are not significantly different (p> 0.05)

CON = Control, EAE = ehuru antioxidant extract, AT = α- tocopherol , EAE/AT = Blend of EAE and AT

Table 4.11: Effect of different concentration on the P anisidine value of actively packaged peanut oil.

SAMPLES	P anisidine value of peanut oil
0%EAE	27.64± 1.05[a]
1% EAE	21.61± 1.64[b]
2.5%EAE	18.84± 1.24[bc]
5%EAE	16.65± 1.30[c]
LSD	**3.6**

Values are the means of duplicate determinations

a,b.... means with the same superscript inthe same column are not significantly different (p> 0.05)

CON = Control, EAE = ehuru antioxidant extract.

Table 4.12: Effect of concentration on the PV of actively packaged peanut oil.

SAMPLES	PV(meq O_2/ kg oil) of peanut oil
0%EAE	12.29± 0.90[a]
1% EAE	9.84± 1.74[b]
2.5%EAE	7.85± 1.13[c]
5%EAE	7.27± 1.69[c]
LSD	**1.85**

Values are the means of duplicate determinations

a,b.... means with the same superscript in the same column are not significantly different (p> 0.05)

CON = Control, EAE = ehuru antioxidant extract.

4.2 DISCUSSION

4.2.1 Phenolic components identified in Ehuru extract

Table 4.1 illustrates the 17 major phenolic compounds identified by GCMS, whose constituents include: 2-Acetylcyclopentanone, Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, acetate, Linalool, γ-muurolene,Alpha-phellandrene, D-Limonene, etc.

4.2.1.1 Bicyclo [3.1.0] hexan-3-ol,

Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, acetate (sabinyl acetate) molecular formula $C_{12}H_{18}O_{2.}$ Molecular number of 194. Its compound nature is monoterpene alcohol. Monoterpenes are class of terpenes that consist of two isoprene units and have several monoterpene derivatives. They have antibacterial and antioxidant activity (Mezza et al., 2018).

4.2.1.2 Linalool.

The IUPAC name of linalool is 3, 7-Dimethyl-1, 6-octadien-3-ol that belongs to the family of Monoterpenes. These are compounds contaning a chain of two isoprene units. Refered to two enantiomers of a naturally occurring terpene alcohol found in many flowers and spice plants. Linalool and linalool-rich essential oils are known to exhibit various biological activities such as antimicrobial, anti fungal, anti-inflammatory, anticancerand antioxidant activities. Linalool is also a key compound for industrial production of fragrance chemicals such as geraniol, nerol, citral. Linalool has shown antioxidant and antibacterial properties in several studies (Baschieri, Ajvazi, and Tonfack, 2017; Aytac, Yildiz, Kayaci-Senirmak, Tekinay , 2017; Seol, Kang, Lee, and Seol, 2016). It was reported that , linalool inhibited growth of model Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria to a great extent. Also, characteristics of liquid linalool have been preserved in a solid nanofiber form

and designed CD/linalool-IC-NFs confer high loading capacity, enhanced shelf life and strong antibacterial activity.

On biological activity of linalool,The highest radical scavenging activity was associated with the linalool-eugenol chemotypes, with the highest activity observed in those with highest content of eugenol.

4.2.1.3 Gamma muurolene (7-methyl-4-methylene-1-propan-2 yl)

This is a group of isomeric aromatic hydrocarbons that are classified as terpenes with molecular formula ($C_{15}H_{24}$). They each have the same molecular formula and carbon framework, but they differ in the position of carbon-carbon double bonds. Functional uses include flavor and fragrance agents. Their activity include protecting plants from bacteria, fungus and other environmental stress (Baschieri, Ajvazi, and Tonfack, 2017).

4.2.1.4 D-limonene

The phenolic compound D- limonnene is a cyclic monoterpene. It is the major component in oil of citrus fruit peels. It is a hydrophobic compound and has plasticizing effect, when a phenolic compound D-limonene (plasticizer) was incorporated in PLA polymer it improved the mechanical and barrier properties of the package, plasticizing and improving the crystallinity of films works of Arrieta *et al*., 2013; Arrieta *et al.,* (2014) and Sanchez-Garcia, Gimenez, & Lagaron, (2008) correlates this findings

4.2.2 Mechanical Properties (Tensile stress)

A good flexibility in other to prevent breaking during food packaging is very important for polymer films. The effect of Ehuru concentration and its AT blend on the mechanical properties was evaluated by tensile stress (TS) and Elongation at break (EAB) of the films.

Table 4.2 shows tensile stress (TS) and elongation at break (EAB) of natural antoxidant films prepared with EAE, AT and blend of EAE and AT. The tensile

strength is the measurement of maximum strength of a film to withstand applied tensile stress. Results show that films incorporated with either EAE or AT showed significant decrease in TS compared to the control film (Pure PSF) ($p < 0.05$). For the EAE incorporated films, the tensile strength decreases from 8.132MPa to 7.426 MPa as the percentage composition increases from 0-5%. The film containing (5%) AT showed very pronounced reduction of tensile strength (5.636MPa) compared to all concentration of EAE investigated. The blend of AT/EAE (5%) improved the tensile strength from 5.636MPa to 6.405MPa.

A good flexibility to prevent breaking during food packaging is very important for polymer films. From Table 4.2, the effect of *Monodora myristica* antioxidant extract concentration and it's AT blend on the tensile strength and elongation at break show that there is a significant decrease in tensile strength of the films incorporated with either EAE or AT compared to that of pure PS film without any antioxidant ($p < 0.05$). The results compares favourably with the works Arrieta et al., (2014).

The EAB value of the pure PSF (control), 2%,; however, the EAE incorporated film values were significantly increasing ($p < 0.05$), as concentration increases (2.353% - 3.221%) This effect was also more pronounced in films containing AT and also with the blend (EAE/AT) films which displayed significantly ($p < 0.05$) reduced values of EAB of 1.70 and 1.75% respectively. The presence of EAE improved the EAB of the film and this is in agreement with the findings of the following researchers Arrieta et al., (2014); Ma *et al.*, (2017); Martinez-Pardo *et al.*, (2017); Noronha *et al.*, (2014b); Siripatrawan & Vitchayakitti, (2016). To avoid breaking during processing and use, films for packaging require high flexibility.

The flexible behaviour may have been caused by the hydrophilic components present in the Ehuru (D-limonene).This also suggests that the plasticizing effect of the EAE could have caused intermolecular interactions between the extract and

PSF making the film relatively flexible which correlates with the findings of Noronha *et al.*, (2014).

Fig 4.2 also shows that the antioxidant level of the active films increases as concentration of the EAE incorporated increases.A similar result was reported by Politeo, Juki, & Milo (2006) that antioxidant activity of *Monodora myristica* increase as concentration increases.

4.2.3 Barrier Property of the Active films

Table 4.3 shows the barrier properties of the active films. The barrier properties of the active films were measured using Oxygen as gas permeant while the transmission pressure was 90cmHg at operating temperature of 25^{o}C. The permeation rate of the gas in the pure PSF was recorded to be 0.1256 cm^3/s. The rate of permeation of the gas in the active films was found to be reducing significantly as concentration of EAE incoporated in the film increased. The 5% EAE produced a film with the highest barrier to the gas having a volumetric flow rate of 0.0277 cm^3/s and permeability of 2.244 $[mL(STP)cmcm^{-2}S^{-1}(cmHg)^{-1}]$. The blend of EAE /AT produced films with lesser barrier hence more porous having volumetric flow rate of 0.1079 cm^3/s; permeability of $2.1228x10^{-6}$ $[mL(STP)cmcm^{-2}S^{-1}(cmHg)^{-1}]$. However, only AT incorporated active film was found to have the least barrier to gas, having flow rate of 0.2209cm^3/s and highest permeability of $3.5791x10^{-6}[ml(STP)cmcm^{-2}S^{-1}(cmHg)^{-1}]$. Moreover, increasing the thickness of AT film did not impede the permeability rate, this was shown when AT film with double the thickness of 5%EAE produced far less barrier and highly permeable film compared to the 5%EAE film according to Guo, Lee, & Tomasko, (2008), foam structure influences barrier properties of films.

4.2.4 Thermal Analyses of the Active films

Table 4.4 shows the thermal transitions of the active films. Results revealed that the transition temperature (T_g) of EAE active film is 63.85^{o}C while the T_g of all

the active film samples are not significantly different ($p > 0.05$) from one another (62°C). T_g is the temperature at which a material undergoes a structural transition from an amorphous solid (glass) state to a more viscous rubbery state. Below Tg, films are rigid and brittle, where as above it films become flexible and pliable. The melting point, Crystallisation temperature (T_c) and degradation temperature of all the active fims are significantly different ($p > 0.05$) from one another with the control film (pure PSF) having melting point of 230 °C. However, EAE active film had a reduced melting point temperature of 210 °C compared to the pure film. On the other hand, AT active firm recorded a higher melting temperature of 247 °C this is because AT is a heat stable phenolic compound and it is in agreement with report of (Siró *et al*., 2007).However, its blend (EAE/AT) had a reduced melting temperature of 237°C. The addition of either EAE or AT incresed the degradation temperature significantly. However the blend experienced a reduction in the degration temperature of 257.6°C. This shows that EAE is not a heat stable compound compared to AT. The addition of the antioxidant EAE, AT or the blend to the filmsignificantly reduced the crystallisation temperature of the pure film from 192.6^{0}C to 279.5°C, 284.5°C and 257.6°C respectively.

4.2.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Antioxidant Active Films:

The FT-IR spectra of the pure PSF and the EAE antioxidant active film were compared in Figure 4.2, 4.3, and 4.4 to confirm the presence of the functional groups of EAE. The functional groups in the films are active components present in EAE, and they are based on the peak values in the region of IR radiation. When the films were passed through the FT-IR, the functional groups of the components were separated based on its peak ratio. The results of FT-IR analysis confirmed the presence of esters and amides (Fig 4.2, 4.3, and 4.4) functional groups with absorption peaks of 1736.9cm^{-1} for esters and 1684.8cm^{-1}for amide. C-O

asymetric stretch of 1293.4 The O-H aliphatic and aromatic stretch is in the absorption band of 2967cm$^{-1.}$ The absorption peaks of 3526,3302.4 and 3697.5 cm^{-1} in the spectrum of Fig 4.3 and Fig 4.4 are assigned to the N–H out of plane vibration of the amine with vibrational range of

3300-3450 cm^{-1}. The peak of 3697 shifted from the range. The result is in line with the observation of Martinez-Pardo *et al.*, (2017). The results confirmed the presence of EAE in the polymer. The amine group could be as a result of the presence of the acetamide compound in the EAE. Fig 4.4 in the absorption band of 1736.9 cm-^{1} showed the presence of an ester group with the C=O Stretch. The band is in the vibrational range of 1750-1725 cm-^{1}and it also showed the presence of aromatic compound in the EAE. The pure PSF has no absorption band beyond 3063.9cm-^{1} as shown in Fig 4.2, showing that presence of band beyond this level could only be EAE or AT

4.2.6 SEM Morphology of the Antioxidant Active Film.

The morphology of the antioxidant films analysed by Scanning Electron Microscopy (SEM) is shown in Fig. 4.5A –F. The micrograph of the pure PSF film as shown in Fig 4.5a revealed a dense, grainy and tiny porous surface. It was observed that the pores reduced as concentration of the EAE added increased from (0% - 5%). And thus, a sealing across the porous film was observed as shown in Fig 4.5B-D. Incorporation of 5% concentration EAE, produced very smooth and homogenous surface film without apparent phase separation as shown by the compactness with little or no noticeable optical pores observed in Fig 4.5D. This observation could be as a result of crosslinking between the components of the EAE and the PSF, that resulted in reinforcement (Arrieta *et al.*, 2015); and this could have caused the sealing process. Similar observation of Grainy and porous surface were noticed on PSF incorporated with blend of EAE/AT as shown in Fig 4.5E. This observation was more pronounce with only

AT incorporated PSF (Fig 4.5F) which showed larger porous structure in comparison to EAE/AT. The incorporation of EAE produced better distribution of EAE phase in the film matrix but with the addition of the blend AT/EAE or AT to the PSF , produced poor dispersion causing discontinuous phase within the polymer matrix. It is suggested that AT films may have behaved differently because of hydrophobic nature of AT. A homogeneous dispersion of hydrophobic AT into polymeric network of PSF could have increased the spacing between macromolecule chains, and this could have reduce the ionic and hydrogen bonding between the chains thereby inducing the development of structural discontinuities in the films. As shown in in the micrographs Fig 4.5E and F

The modified PSF polymer is in agreement with the findings of Arrieta *et al*., 2013 and Arrieta *et al.,* (2014), when a phenolic compound, D-limonene (plasticizer) was incorporated in PLA polymer.

Hydrophilic substances like the EAE on the other hand, migrates to the PSF voids thereby acting as plasticizer and causing a seal. This is in agreement with the report of Pedrielli *et al.,* (2001).

4.2.7 Oxidative Stability of EAE actively stored peanut oil

Table 4.5 shows the result of the peroxide value (meq O_2/ kg oil) of peanut oil packaged with polysulfone film incorporated different antioxidants. All treatments with or without EAE or AT showed no changes within the first one week of storage. The PV increased slightly within the first 4 weeks after which there was exponencial increase up to the 6th week of storage. It was observed that the control recorded highest maximum level of PV (18.22 meq/kg) before a decrease, and this decrease could be as a result of the decomposition of the unstable peroxide to secondary products (Coupland & McClements, 1996). This is in agreement with the report of Pereira De Abreu *et al*.,(2011) reported that when the maximum level of PV was reached the value decreased as a result of

lower substrate availability and instability of peroxide molecules which easily decomposes. This decrease of imerging peroxides towards the last stage of oxidation was also observed by Jongjareonrak, Benjakul, Visessanguan, & Tanaka, (2008). The maximum level of PV for AT and EAE/AT blend were both on the 5th and 7th week of storage (12.70±0.63,12.83±0.84) meq/kg respectively. Showing that they both stored more than the control sample. However EAE packaged oil recorded maximum PV value at the 6th week of storage. It was also observed to have constant PV values that were not significantly different ($p > 0.05$) during the 2nd to 4th week of storage. This shows that the EAE was able to halt oxidation within this period which led to the lowest value of PV recorded at the end of storage. This indicates that the antioxidant EAE could have scavenged the initial peroxyl radical (peroxides) produced, thereby inhibiting radical induced oxidation. (chain breaking activity of H atom transfer) in line with the findings of Baschieri *et al*., (2017); Bentayeb *et al*., (2007); Foti & Ingold, (2003). It was confirmed that the scavenging activity could be as a result of highpolyphenol content shown by the presence of all the monoterpene components present in the EAE which possessed significant antioxidant activities that could be responsible for slowing down the autoxidation of methyl linoleate by oxidation mechanism. Terpenes causes a faster oxidative chain-termination due to the generation of hydroperoxyl radicals (Foti and Ingold , 2003).

The forestalled radicals up to the 4^{th} week of storage made the peanut oil packaged in EAE film to record the lowest maximum PV level (9.5±0.65 meq/kg) at the 6^{th} week of storage and also at the end of nineth week of storage (9.82±1.50meq/kg).

Results showed that there was no significant diference ($p > 0.05$) between the PV value of peanut oil stored with EAE/AT and AT films. The PV in the sample packaged with control film (without antioxidants) was significantly higher

($p<0.05$) than all the other samples. This confirms the fact that antioxidants act by inhibiting the mechanism of lipid auto- oxidation of free radicals. This ability was due to the phenolic structure within the

structure EAE. Phenolic substances (antioxidant) act as free radical acceptors and are able to hinder rate of oxidation of the peanut oil. molecular (Mattia, Sacchetti, Mastrocola, & Pittia, 2009)

Table 4.6 gives the results of p-anisidine value (p-AV) of peanut oil packaged with different active films.The results showed that p-AV of all the samples excluding the EAE actively packaged samples were significantly increasing ($p<0.05$) with storage time.The oil packaged with the control film (without any antioxidant) was more susceptible to oxidation than oil samples packaged with active films. This is indicated by its higher rate of production of p-AV especially between the 4th and 5th week of storage (23.45±0.25 and 40.27±0.44) and between the 8th and 9th week (43.25±0.30 and 49.37±0.24) respectively. This led to highest p- AV (49.37±0.24) at the end of storage period. However the oil packaged with EAE film showed no significant diference ($p> 0.05$) between the third and fourth week of storage (13.31±0.01, 14.63±0.41) and between the 6th and 7th week of storage (24.21±0.04, 24.25±0.56). This shows that within these period oxidation was suppressed.

At the end of 9^{th} week of storage, oxidation of peanut oil packaged in EAE film gave the lowest p-AV of 30.45±0.15 in comparism to the other films. This shows that EAE film had the capacity to suppress the rate of oxidation of the peanut oil in contact with it. All the three peanut oil samples packaged with the active films had significant increase of p-AV between the 7th and 8^{th} week of storage, indicating the reaction of p-anisidine with the produced aldehydes.

Hydroperoxides are unstable at storage, therefore they decompose to give secondary oxidation products that are *p*-anisidine-reactive. This finding was also noted by Moigradean, Poiana, & Gogoasa (2012).

No significant difference ($p > 0.05$) was observed between oil in AT and blend of EAE/AT films. Comparing EAE with the blend showed that AT reduced the effectiveness of EAE which could be as a result of their incompatibility. AT being hydrophobic while EAE is hydrophilic.

Table 4.7 shows the peroxide values (PV) over storage time of peanut oil packaged with different concentration of EAE films. The results showed that the PV of all the samples were increasing with time of storage. The PV of the control film (0% EAE film) over time was significantly increasing more than the rest of the peanut oil samples packaged in EAE films, irrespective of their concentrations. This was because the film had no antioxidative property in it. Generally, Table 4.6 shows that as the concentration of EAE in the film increased the PV of the packaged oil reduced. At the end of storage duration, 2.5% EAE recorded the least PV value of 9.01±0.12 meq/kg compared with the control (0% EAE) with highest PV of 20.50±1.10 meq/kg. The results also showed that the peroxide value of oil stored with control and 1% EAE films increased significantly ($p > 0.05$) from the first week 8.22±0.35 of storage to the 6th week of storage 15 ±0.30. Oil stored with 2.5% EAE film had no significant difference ($p > 0.05$) between the peroxide values recorded in the 2nd and the 3rd week of storage. Oil stored with 5% EAE film, also showed no significant difference between PV recorded in the 2nd, 3rd and 4th week of storage with values of 6.34±0.09, 6.41±0.28 and 6.63±0.44 respectively.

Table 4.8 shows the p-anisidine value of peanut oil packaged in EAE film of different concentrations. The results show that the p-anisidine value of the

peanut oil pakaged in different concentrations of EAE increased significantly ($p < 0.05$) over time.

Table 4.9 shows the effect of different antioxidant on the PV of the actively packaged peanut oil. The results showed that the control film (with no antioxidant) gave the highest PV of 12.29 ± 0.90. The value was significantly different ($p<0.05$) from the results of any of the antioxidant packs. The PV of EAE/AT blend and AT did not show any significant difference ($p< 0.05$) with values of 8.8± 3.07 and 7.35± 2.00 respectively. Rather the combination reduced the potential of EAE by having values of 7.35± 2.00 that was higher and significantly ($p< 0.05$) different from EAE. EAE recorded lowest PV which is significantly different from all the antioxidant packs. This shows that EAE had the highest oil oxidation inhibiting potential.

Table 4.10 shows the effect of different antioxidants on the p-AV of the actively packaged peanut oil. Results showed that their was no significant difference($p> 0.05$) between oil packaged and stored in EAE/AT film and AT film. Mean while oil packaged and stored in EAE film had the least of p-AV value. The control had the highest value of p-AV .

Table 4.11shows the effect of different antioxidant on the p-anisidine value of actively packaged peanut oil. The results showed that oil in EAE package had the least value and is not significantly different ($p> 0.05$)from the p-anisidine value of oil stored with active package of blend of EAE/ AT and oil stored with active package containing only AT. The control had the highest p-anisidine value and it is significantly different ($p< 0.05$) from other oil packaged and stored in the active packages.

Results also showed that there was no significant ($p> 0.05$)difference between oil in 5% EAE package and oil in 2.5% EAE package, even though 5% EAE had the least p-anisidine value of 7.27± 1.69

Table 4.12 showed the effect of concentration on the PV of actively packaged peanut oil. The Results show that oil packaged and stored in 2.5% EAE and 5% EAE were not significantly ($p > 0.05$) different. This indicates that between 2.5 and 5% of EAE active film could effectively be used to mitigate the rate the peanut oil oxidation.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Anantioxidant active packaging system was succesfully developed using ehuru extract. The ehuru antioxidant package produced exhibited more flexible and stronger mechanical characteristic which is a very important property in food polymer package material in order to prevent breakage.

The film manifested a very good scavenging ability which was demonstrated by its considerable capability in delaying oxidation especially during an early stage of oil oxidation, hence its effectiveness in increasing shelf life of oily food.

The mechanical (tensile), microstructure, and thermal properties of the films were significantly influenced by the addition of EAE .

The peroxide and p-anisidine values obtained showed that the films actively protected the peanut oil by delaying oxidation. From the results obtained, concentration betweens 2.5% EAE and 5%EAE film could effectively mitigate lipid oxidation.

The ehuru antioxidant extract film showed an antioxidant activity better than that of alpha tocopherol (AT), a known natural antioxidant used in inhibiting lipid peroxidation.

The incorporationofEAE to thePSF film produced an improved gas barrier package. This produced a better effect compared with AT film with double thickness

5.2 RECOMMENDATION

Further studies on incorporating ehuru into other biopolymers such as polyamide, polylactic acid (PLA), polyethylvinyl acohol, cellulose based polymer, to produce an active film could also be explored.

The use of other spices as active agent on the polysulfone film should also be explored.

Pure compounds like α-phellendrene, p-cymene, γ- muurolene from the Ehuru extracts can also be incorporated into polymer resins.

The antimicrobial effects of films containing ehuru extact or pure compounds from ehuru could also be further investigated.

Shelf live prediction could be carried out, where data on microbial, sensory and chemical anlyses may be combined for adequate prediction. Studies aimed at establishing the type of pure antioxidant agent and optimum antioxidant concentrations to maximize the shelf life, while at the same time maintaining good sensorial characteristics are required.

5.3 CONTRIBUTION TO KNOWLEDGE

- This study has revealed that *Monodora myristica* (ehuru) extract can effectively and successfully be infused into polysulfone polymer as active agent for controlling lipid oxidation.
- The research has shown that Polysulfone polymer can be used as material for active package production; this has not been reported in previous studies.
- The infusion of ehuru extract (EAE) component in the film showed antioxidant and scavenging capabilities to supress lipid oxidation. These

films should be explioted for lipid foods' preservation and shelf-life extension.

- The EAE films produced showed high barrier to oxygen with low volumetric flow rate of 0.0277 cm^3/s and permeability of 2.244x 10^{-7} $[mL(STP)cmcm^{-2}S^{-1}(cmHg)^{-1}]$ compared to the pure film with high permeability of $8.721^{c}x10^{-7}$ $[mL(STP)cmcm^{-2}S^{-1}(cmHg)^{-1}]$ at 25^0C and pressure of 90cmHg

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APPENDIX

Appendix 1: Antioxidative/ radical scavenging property of the active polymer films.

$$\left(\frac{A\,reference - Asample}{Areference}\right)x100$$

0% EAE $A\,reference - Asample/Areference$

$$\left(\frac{2.580 - 2.500}{2.580}\right)x100$$

$$= 3.100\%$$

1% EAE

$$\left(\frac{2.580 - 2.320}{2.580}\right)x100$$

$$= 10.077\%$$

2.5% EAE

$$\left(\frac{2.581 - 1.997}{2.581}\right)x100$$

$$= 22.590\%$$

5% EAE

$$\left(\frac{2.601 - 1.244}{2.601}\right) x100$$

$$= 48.097\%$$

5% AT

$$\left(\frac{2.582 - 1.250}{2.582}\right) x100$$

$$= 51.588\%$$

5% EAE/AT

$$\left(\frac{2.578 - 1.522}{2.578}\right) x100$$

APPENDIX 2: Permeability and flow rate of the active film

A: Flow rate of the Active polymer films

Membrane description	P2 (Bar)	P1(Bar)	$L(cm)$	A (cm2)	T (K)	P (Bar)	dV/dt (cm3/s)
No 3	2.04	0.84	0.014	9.6	299	1.2	0.0593
No 14	2.04	0.84	0.017	9.6	299	1.2	0.1079
No 13	2.04	0.84	0.014	9.6	299	1.2	0.2209
No 6	2.04	0.84	0.005	9.6	299	1.2	0.0463
No 7	2.04	0.84	0.007	9.6	299	1.2	0.0277
No 2	2.04	0.84	0.006	9.6	299	1.2	0.1256

where

P2 = Feed Pressure

P1 = Permeate Pressure

P = Transmembrane pressure

A = Permeation Area

L = Membrane thickness

T = Operating temperature

dV/dt = Volumetric displacement

B: Calculation of Permeability

Permeability $= \left(\frac{Amount\ of\ Gas\ x\ Thickness\ of\ the\ film}{Areax\ Presure\ diference\ across\ the\ film} \right)$

0.0%EAE

$$\left(\frac{0{,}1256x\ 0.006}{9.6x\ 90.0077}\right)$$

$$= 8.721x10^{-7}[\text{mL (STP) cmcm}^{-2}\text{S}^{-1}(\text{cmHg})^{-1}]$$

1.0%EAE

$$\left(\frac{0.0463x\ 0.014}{9.6x\ 90.0077}\right)$$

$$= 7.502x10^{-7}[\text{mL (STP) cmcm}^{-2}\text{S}^{-1}(\text{cmHg})^{-1}]$$

2.5%EAE

$$\left(\frac{0.0593x\ 0.005}{9.6x\ 90.0077}\right)$$

$$= 3.431x10^{-7}[\text{mL (STP) cmcm}^{-2}\text{S}^{-1}(\text{cmHg})^{-1}]$$

5.0%EAE

$$\left(\frac{0.0277x\ 0.007}{9.6x\ 90.0077}\right)$$

$$= 2.244x10^{-7}[\text{mL (STP) cmcm}^{-2}\text{S}^{-1}(\text{cmHg})^{-1}]$$

5.0%EAE/AT

$$\left(\frac{0.1079x\ 0.017}{9.6x\ 90.0077}\right)$$

$$= 2.1228x10^{-6}[\text{mL (STP) cmcm}^{-2}\text{S}^{-1}(\text{cmHg})^{-1}]$$

5.0%AT

$$\left(\frac{0.2209x\ 0.014}{9.6x\ 90.0077}\right)$$

$$= 3.5791x10^{-6}[\text{mL (STP) cmcm}^{-2}\text{S}^{-1}(\text{cmHg})^{-1}]$$

Appendix 3: FTIR *Vibrational bands* of the active Polymer films

A: FTIR v*ibrational bands* for neat PSF Polymer compositions

Wave numbers and Assignment *of Vibrational bands* for 0 % Polymer composition

S/N.	Peak Value (cm^{-1})	Intensity	Range (cm^{-1})	Functional groups and Possible compounds	Assignment
1	693.3-961.7	strong	690-710	Alcohol	O-H bend / C=C Stretch out of plane
2	1103.3, 1237.5	Strong	1035-1260	Alcohols, Aldehydes	C=O stretch / Ar-O-H
3	1323.2	Strong	1000-1323.2	Sulphones, Sulphonamides	S=O
4	1487.2	Strong	1450-1600	Aromatic compounds	C-C=C Asymmetric Stretch
5	1584.1	Strong	1450-1600	Aromatic compounds	–C=O Stretching vibrations
6	2920.1	strong	2854-2924	Methylene -(CH2)*n*	symmetric and antisymmetric -CH_2 stretching vibrations,

B:FTIR *Vibrational bands* for 5 % Ehuru/Polymer composition

Wave numbers and Assignment *of Vibrational bands* for 5% Ehuru / Polymer compositions

S/N.	Peak Value (cm^{-1})	Intensity	Range (cm^{-1})	**Functional groups and Possible compounds**	Assignment
1	693.3-961.7	strong	690-710	Alcohol	O-H bend / C=C Stretch out of plane
2	1103.3 - 1237.5	Strong	1035-1260	Alcohols, Aldehydes	C=O stretch / Ar-O-H
3	1323.2	Strong	1000-1323.2	Sulfones, Sulfonamides	S=O
4	1487.2	Strong	1450-1600	Aromatic compounds	C-C=C Asymmetric Stretch
5	1584.1	Strong	1450-1600	Aromatic compounds	–C=O Stretching vibrations
6	**1736.9**	weak	**1710-1720**	Aldehyde	C=O Stretch
7	**3302.4 – 3697.5**	Strong and broad	**3000-3700**	Amine	N-H, O-H Out of plane

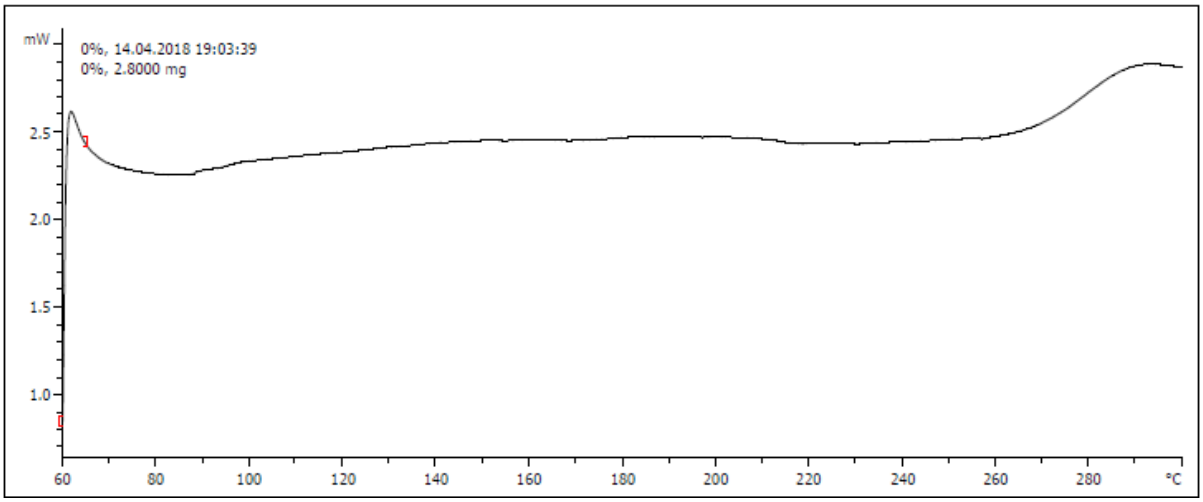
C: *FTIR vibrational bands* for 2.5% Ehuru / 2.5% α –T / Polymer compositions

Wave numbers and Assignment *of Vibrational bands* for 0.25% Ehuru / 0.25% α –T / Polymer compositions

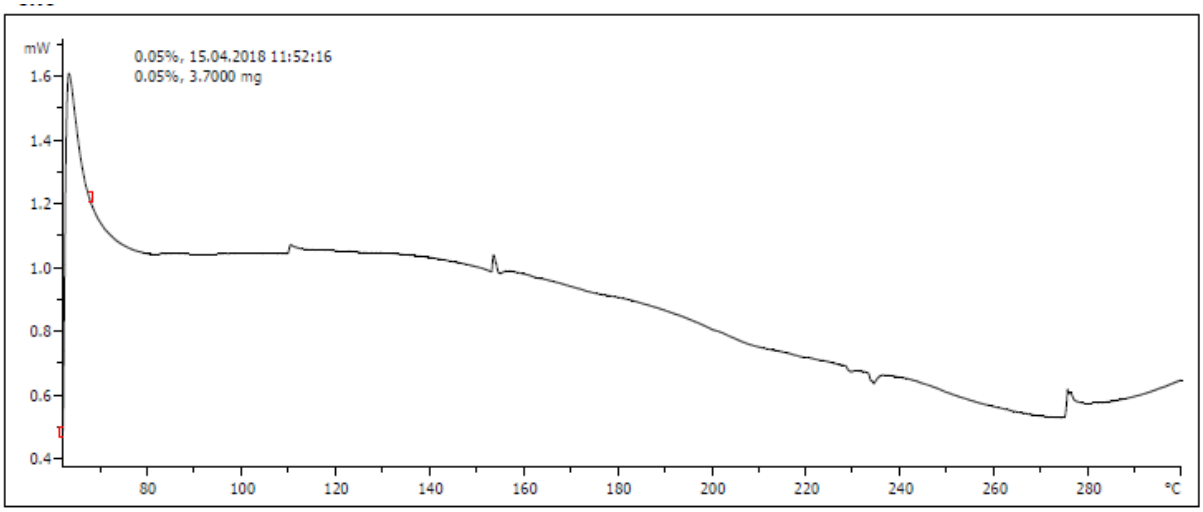
S/N.	Peak Value (cm^{-1})	Intensity	Range (cm^{-1})	Functional groups and Possible compounds	Assignment
1	693.3-961.7	strong	690-710	Alcohol	O-H bend / C=C Stretch out of plane
	738.0	strong	750-720	Methylene -(CH2)*n*	(-CH_2)n-rocking
2	1103.3-1237.5	Strong	1035-1260	Alcohols, Aldehydes	C-O stretch/ Ar-O-H
3	1323.2	Strong	1000-1323.2	Sulfones, Sulfonamides	S=O
4	1487.2	Strong	1450-1600	Aromatic compounds	C-C=C asymmetric Stretch
5	1584.1	Strong	1450-1600	Aromatic compounds	–C=O stretching vibrations
6	1684.8	Strong	1640-1670	Amide	N-R Stretch
7	1736.9	weak	1750-1725	Ester	C=O Stretch
8	3526	Broad peak and weak	3000-3700	Amine	N-H, O-H Out of plane

APPENDIX 4: DSC Curves of the Antioxidative Active films

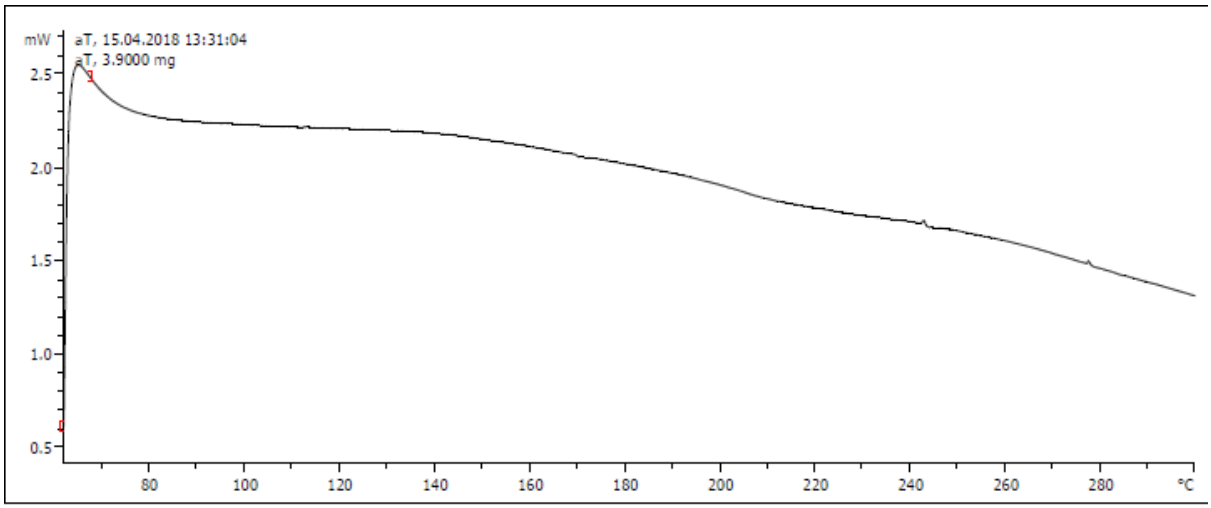
A: 0% EAE



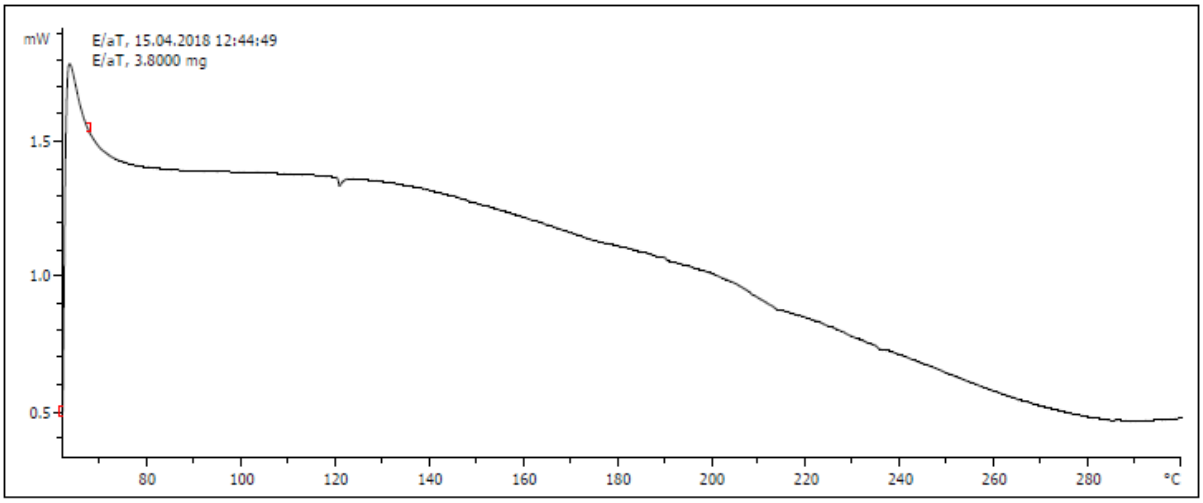
B: 5%EAE



,6C: AT

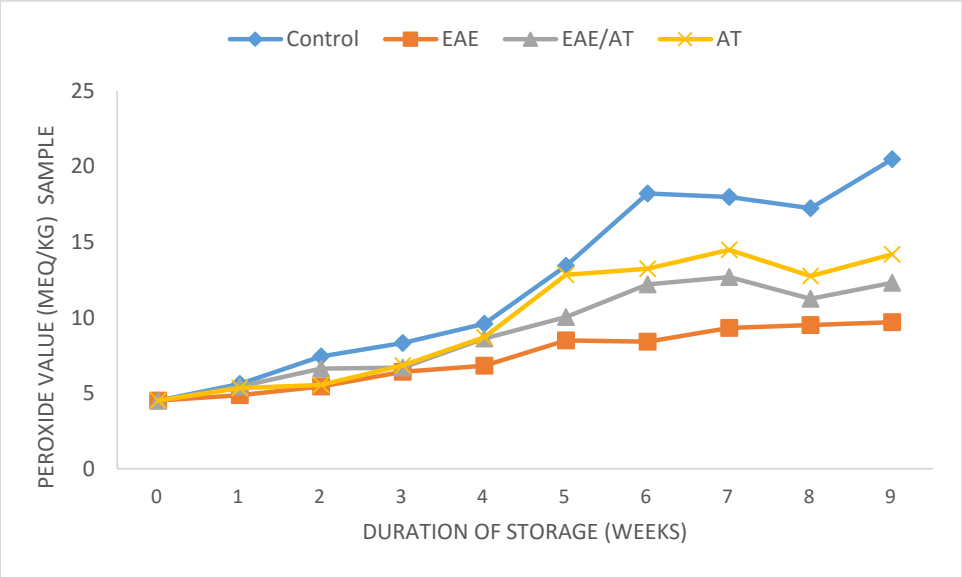

mW
aT, 15.04.2018 13:31:04
aT, 3.9000 mg
2.5
2.0
1.5
1.0
0.5
80
100
120
140
160
180
200
220
240
260
280
°C

D: E/AT

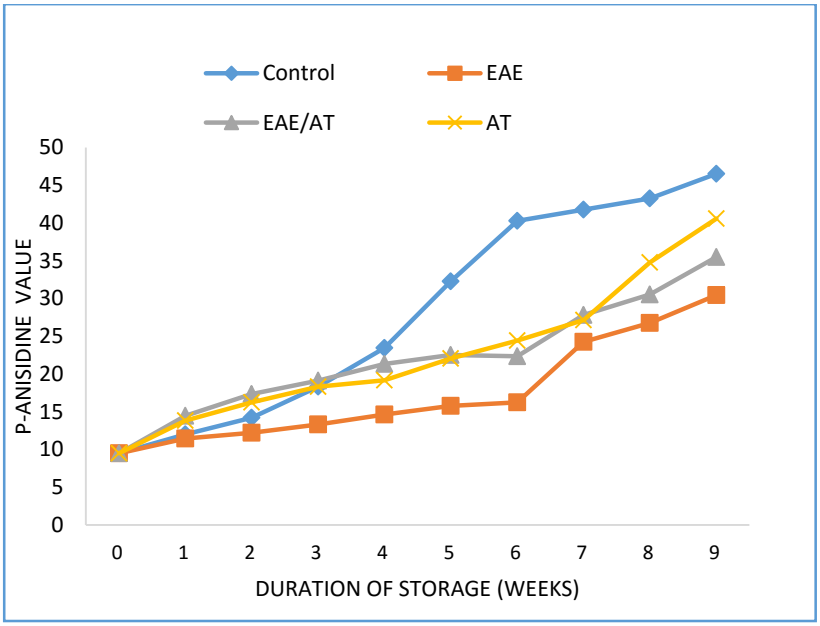

mW
E/aT, 15.04.2018 12:44:49
E/aT, 3.8000 mg
1.5
1.0
0.5
80
100
120
140
160
180
200
220
240
260
280
°C

APPENDIX 5: Graphical representations of oxidation of actively packaged peanut oil

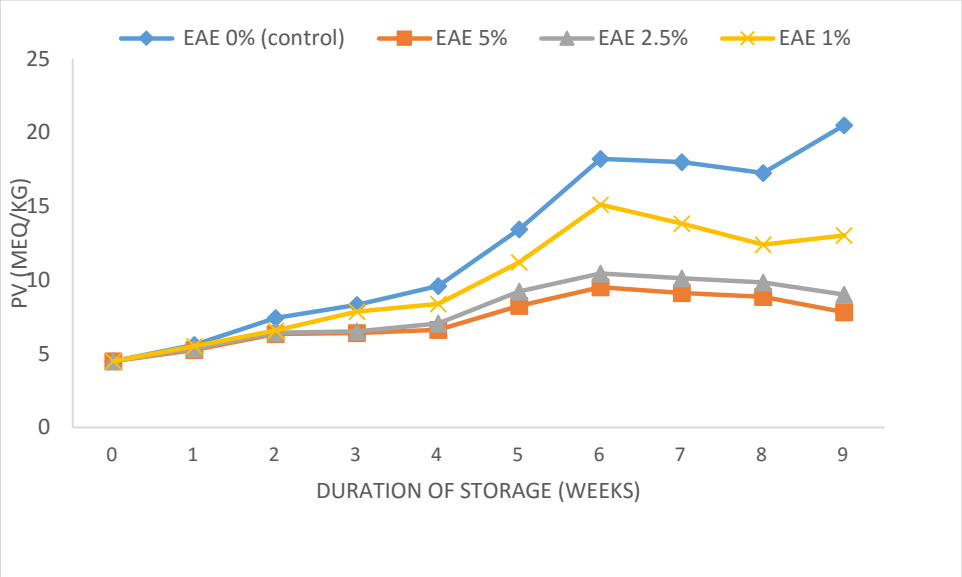
A: PV of peanut oil packaged with film containing different antioxidants



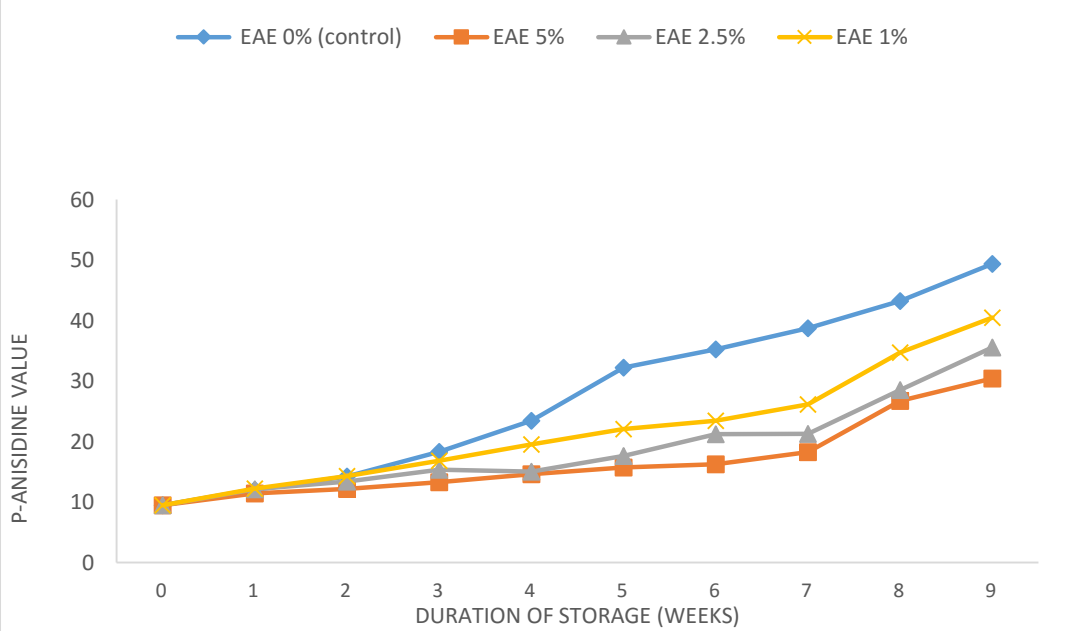
B: p- anisidine value of peanut oil packaged with films containing different antioxidants



C:PV of peanut oil packaged with films containing different concentration of EAE

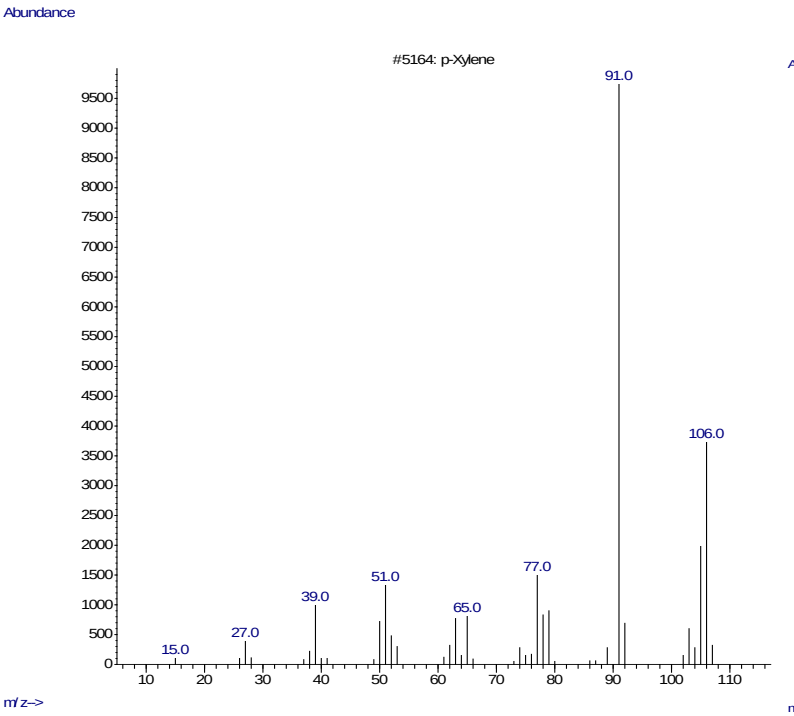


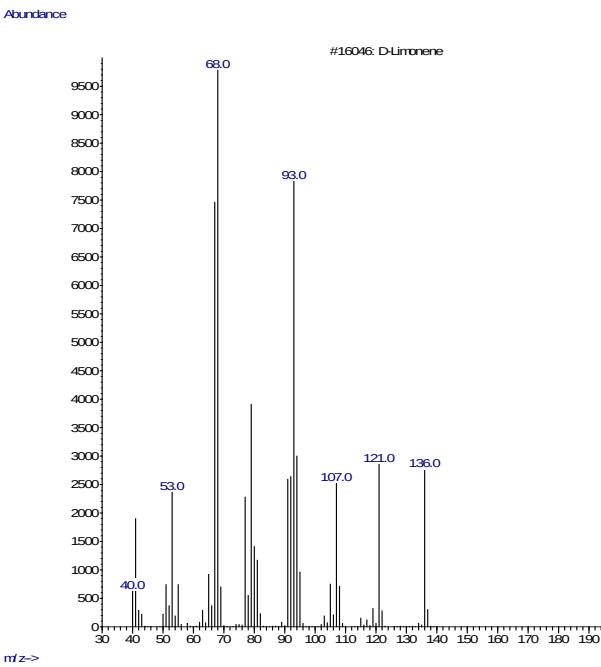
D:p-anisidine value of peanut oil packaged with film of different concentration of EAE

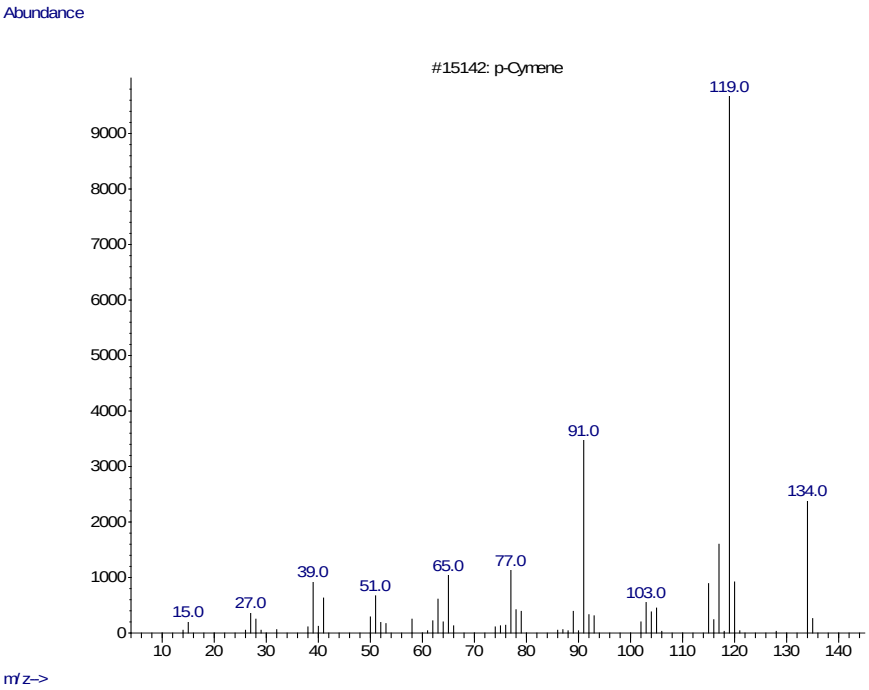


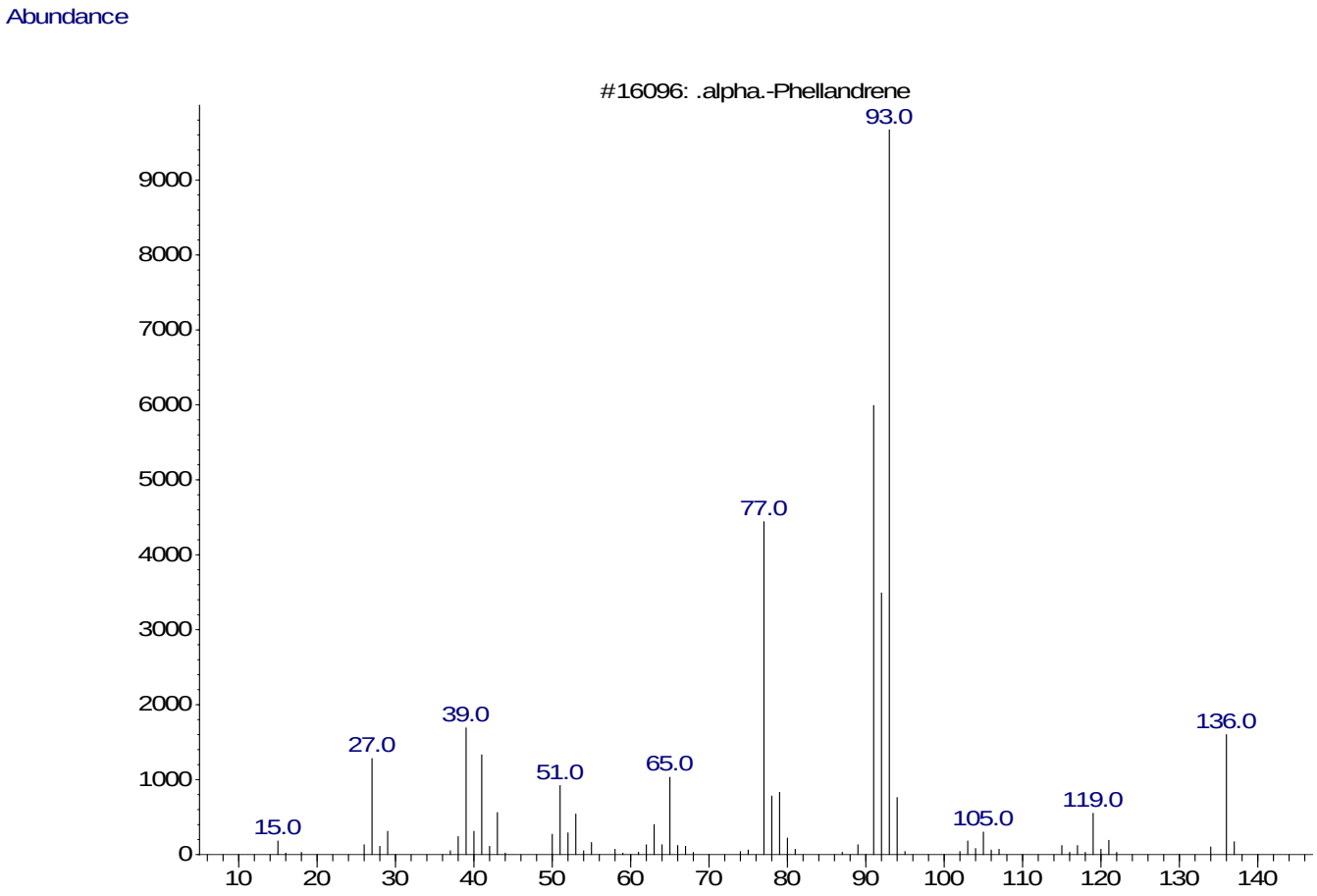
APPENDIX 6

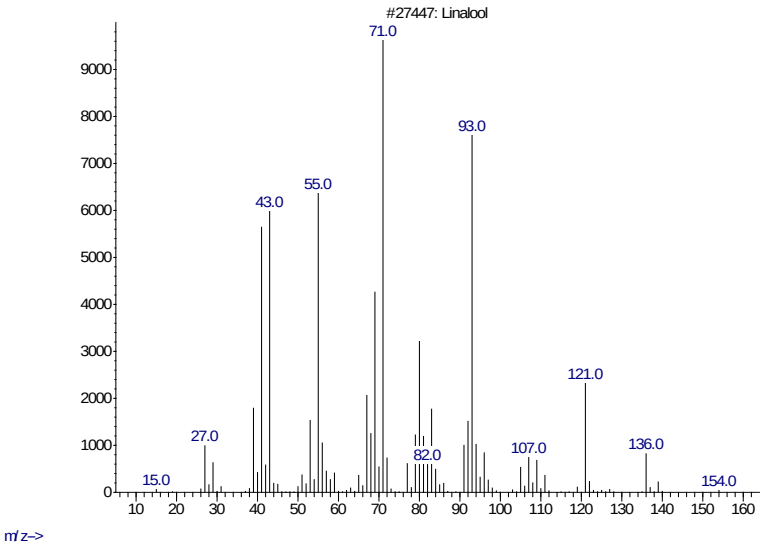
The GCMS spectra of ethanolic extract of Ehuru



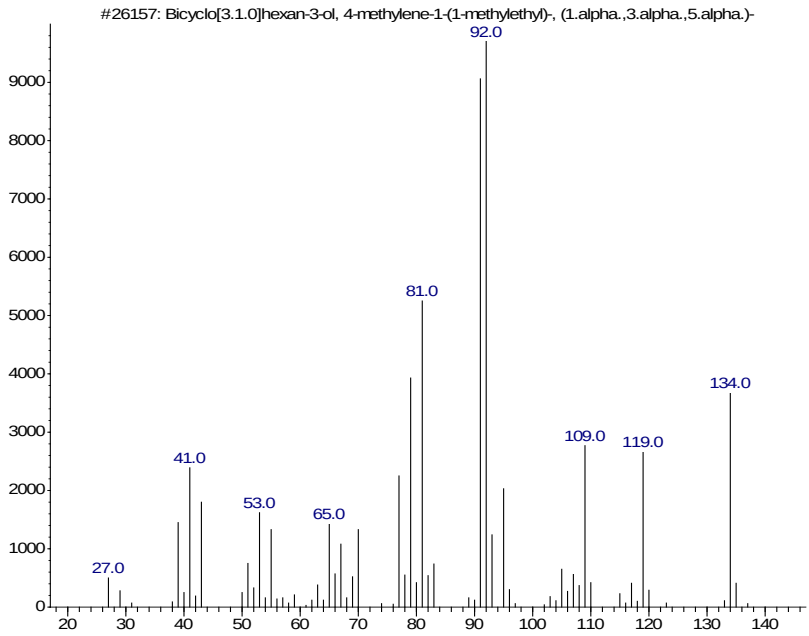




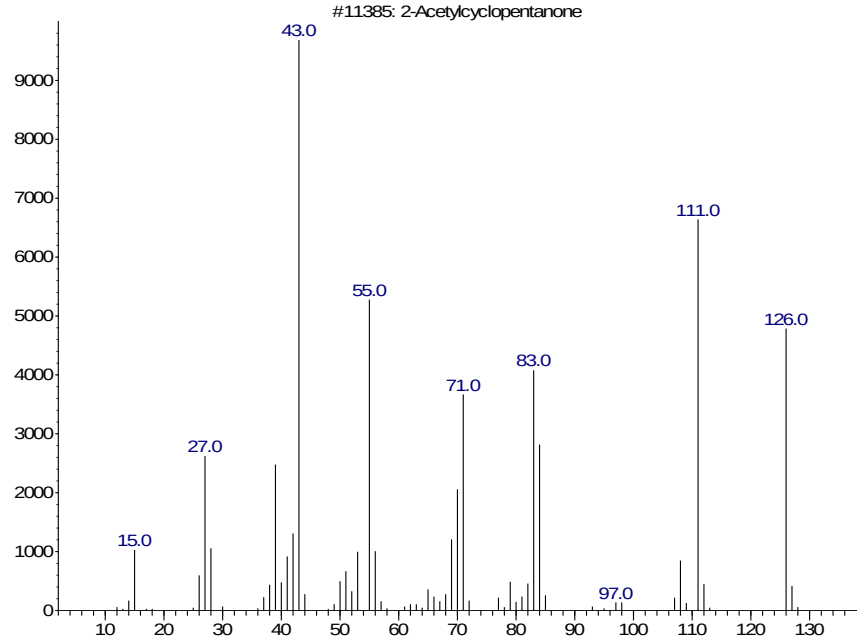

Abundance
#16096: .alpha.-Phellandrene
93.0
77.0
39.0
27.0
51.0
65.0
136.0
119.0
105.0
15.0
m/z-->


Abundance
#27447: Linalool
71.0
93.0
55.0
43.0
121.0
27.0
107.0
136.0
82.0
15.0
154.0
m/z-->

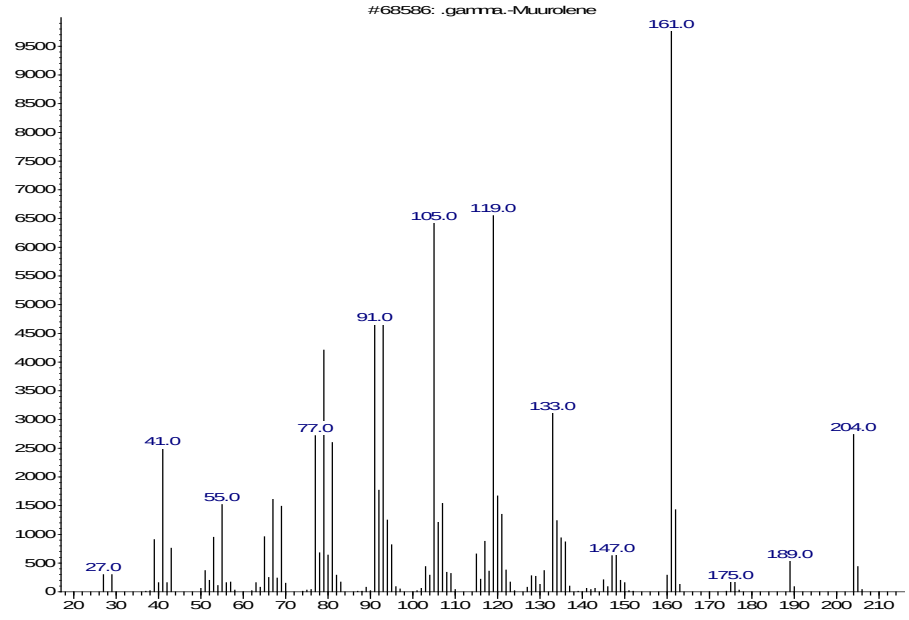
Abundance



Abundance

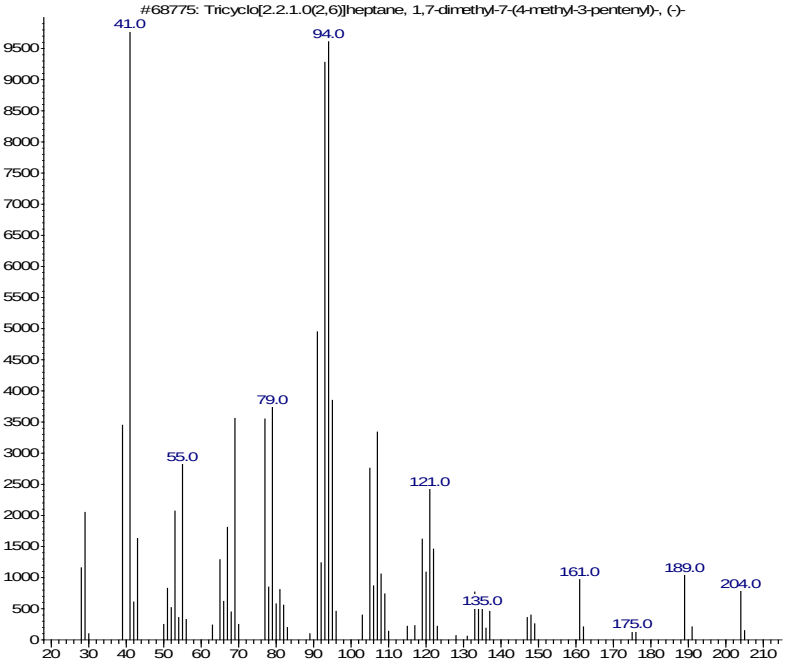


Abundance



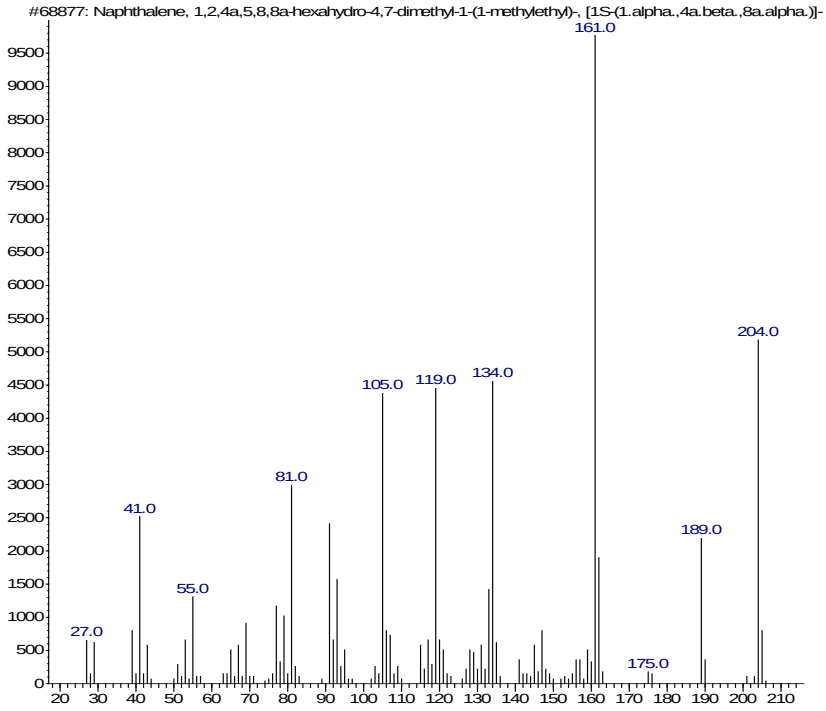
m/z-->

Abundance



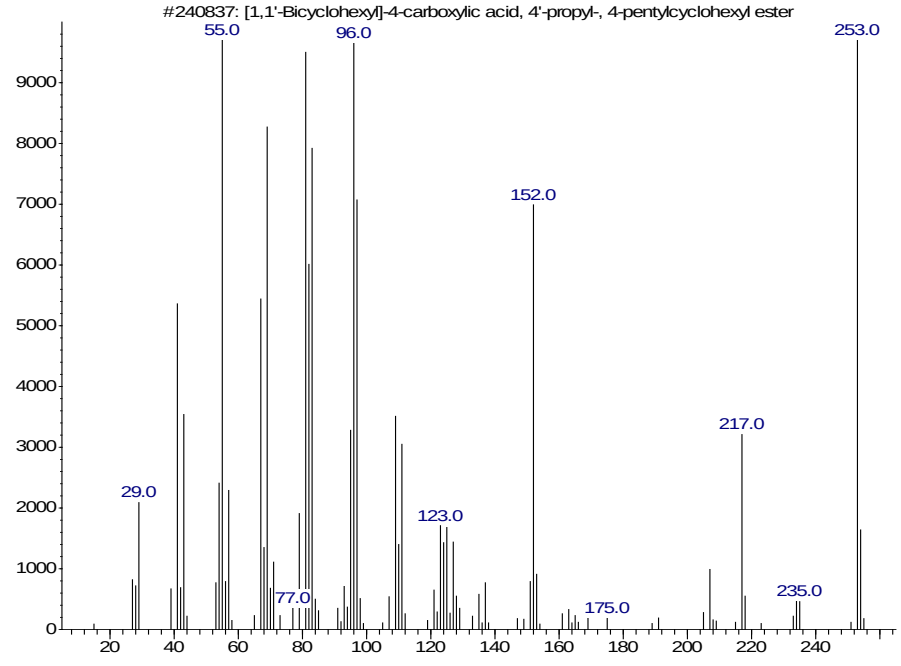
m/z-->

Abundance



m/z-->

Abundance



m/z-->

APPENDIX 7: Anova of oxidation analyses of the actively packaged oil.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	678.8902	3	226.2967	14.6875	7.26E-06	2.960351
Columns	2864.662	9	318.2957	20.65858	6.66E-10	2.250131
Error	416.0008	27	15.40744			
Total	3959.553	39				

LSDR	5.694973
LSDC	3.601817

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	
0%EAE	10	122.87	12.287	34.78002	5.897459
1% EAE	10	98.39	9.839	14.00594	3.742452
2.5%EAE	10	78.49	7.849	4.55481	2.1342
5%EAE	10	72.67	7.267	2.871646	1.694593
0	4	18	4.5	0	0
1	4	21.74	5.435	0.025233	0.15885
2	4	26.77	6.6925	0.250692	0.500691
3	4	29.11	7.2775	0.917492	0.957858
4	4	31.66	7.915	1.8183	1.348444
5	4	42.12	10.53	5.281667	2.298188
6	4	53.27	13.3175	16.66923	4.082796
7	4	51.06	12.765	16.29817	4.037099
8	4	48.34	12.085	14.0939	3.754184
9	4	50.35	12.5875	32.77409	5.724866

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	154.5074	3	51.50246	12.65545	2.37E-05	2.960351
Columns	396.0329	9	44.00365	10.8128	6.71E-07	2.250131
Error	109.8789	27	4.069589			
Total	660.4192	39				

LSDR	2.926859
LSDC	1.851108

APPENDIX 8: Anova of barrier properties

L (CM)

SAMPLE	1ST	2ND
0EAE	0.006	0.006
1%EAE	0.01	0.018
2.5%EAE	0.0048	0.0052
5%EAE	0.0067	0.0073
5%EAE/AT	0.0015	0.0019
5%AT	0.013	0.015

LSD

0.00585387

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	0.012	0.006	0
Row 2	2	0.028	0.014	0.000032
Row 3	2	0.01	0.005	8E-08
Row 4	2	0.014	0.007	1.8E-07
Row 5	2	0.0034	0.0017	8E-08
Row 6	2	0.028	0.014	0.000002

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000251	5	5.03E-05	8.783343	0.009893	4.387374
Within Groups	3.43E-05	6	5.72E-06			
Total	0.000286	11				

FLOW RATE

SAMPLE	1ST	2ND
0EAE	0.125	0.1262
1%EAE	0.046	0.0466
2.5%EAE	0.0593	0.0593
5%EAE	0.027	0.0284
5%EAE/AT	0.107	0.1088
5%AT	0.2218	0.22

LSD

0.00226036

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	0.2512	0.1256	7.2E-07
Row 2	2	0.0926	0.0463	1.8E-07
Row 3	2	0.1186	0.0593	0
Row 4	2	0.0554	0.0277	9.8E-07
Row 5	2	0.2158	0.1079	1.62E-06
Row 6	2	0.4418	0.2209	1.62E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.050154	5	0.010031	11754.77	6.98E-12	4.387374
Within Groups	5.12E-06	6	8.53E-07			
Total	0.050159	11				

PERMEABILITY

SAMPLE	1ST	2ND
0EAE	8.72	8.722
1%EAE	7.5	7.504
2.5%EAE	3.43	3.432
5%EAE	2.24	2.248
5%EAE/AT	0.2122	0.21236
5%AT	0.35791	0.35791

LSD

0.00662723

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	17.442	8.721	2E-06
Row 2	2	15.004	7.502	8E-06
Row 3	2	6.862	3.431	2E-06
Row 4	2	4.488	2.244	3.2E-05
Row 5	2	0.42456	0.21228	1.28E-08
Row 6	2	0.71582	0.35791	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	130.3594	5	26.07188	3554223	2.53E-19	4.387374
Within Groups	4.4E-05	6	7.34E-06			
Total	130.3595	11				