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Determination of the antioxidant activity of surface treated PET films coated with natural extracts

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SCHOOL OF SCIENCES AND ENGINEERING

DEPARTMENT OF CHEMISTRY

DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF SURFACE TREATED PET FILMS COATED WITH NATURAL EXTRACTS

A Thesis Submitted to

The Food Chemistry Master's Program

In partial fulfilment of the requirements for

the degree of Master of Science

By

Hebatullah H. Farghal

Under the supervision of

Dr. Mayyada El-Sayed
Dr. Michail Kontominas

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The American University in Cairo

Determination of the antioxidant activity of surface treated PET films coated with natural extracts

A Thesis Submitted by

Hebatullah Hassan Mostafa Farghal

To the Food Chemistry Graduate Program

July, 2016

In partial fulfillment of the requirements for

the degree of Master of Science

Has been approved by

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I

TABLE OF CONTENTS

Dedication

To my parents, grandmother and my new family…..

Acknowledgements

ABSTRACT

Food oxidation is one of the major concerns that food producers and consumers face. In that regard, we are developing antioxidant polyethylene terephthalate (PET) film that could be employed for packaging fish products. Commercial PET films were surface treated and subsequently coated with either rosemary or clove extracts. Surface treatments involved a) corona treatment and b) chemical modification which are industrial means of surface treatment; Plasma treatment, at laboratory scale, was also used. Radical scavenging activities (RSA) of both pure plant extracts and coated film extracts were measured using the 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) method. Treated films coated with rosemary showed %RSA of 25.6%, 22.4% and 24.1% pertaining to plasma, chemical modification and corona treated films at 1402 ppm respectively and AE values of 0.35, 0.26 and 0.28 respectively, while pure rosemary extract showed %RSA of 16.03% and AE value of 0.19. As for the treated films that were coated with clove, it showed %RSA of 25.0%, 25.22% and 25.15% for plasma, chemical modification and corona treatments at 1402 ppm respectively and AE values of 0.31, 0.32 and 0.33 respectively, while the plant extract showed %RSA of 47.62% and AE value of 0.72. Thiobarbituric acid (TBA) antioxidant test was also performed on fish muscle wrapped in all types of films which all showed a remarkable decrease in the degree of fish oxidation ranging between 50 to 80%. All TBA tests were terminated on day 6 of storage which is in accordance with the end of the product microbiological shelf life, where TVC reached the borderline of 7 log cfu/g. Contact angle measurements confirmed that chemically modified films have the highest adhesion power followed by corona then plasma treated films. Scanning electron microscopy (SEM) results supported contact angle measurements where chemically modified films showed the roughest surface followed by corona then plasma treated films. X-ray photoelectron spectroscopy (XPS) measurements also revealed that chemically modified films resulted in the formation of a higher concentration of oxygen containing functional groups on the PET surface as compared to other treatments, indicative of the highest surface adhesion capacity of the chemically modified film. Finally, the oxygen permeability of chemically modified PET films was the same as untreated films which is an indication that treatment did not affect film barrier properties.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADHD: Attention Deficit Hyperactivity Disorder
BHA: Butylated Hydroxyl Anisole
BHT: Butylated Hydroxyl Toluene
DPPH: 2,2- Diphenyl-1-picryl-hydrazyl
EFSA: European Food Safety Authority
FDA: Food and Drug Administration
FRAP: Ferric Reducing Ability of Plasma
FTIR: Fourier Transform Infra-Red
HDPE: High Density Polyethylene
HIPS: High Impact Polystyrene
HPMC: Hydroxy Propyl Methyl Cellulose
LDPE: Low Density Polyethylene
LLDPE: Linear Low Density Polyethylene
PLA: Polylactide
PVdC: Polyvinylidene Chloride
PVDF: Polyvinylidene Difluoride
PS: Polystyrene
OPP: Oriented Polypropylene
ORAC: Oxygen Radical Absorbance Capacity
PET: Polyethylene terephthalate
RSA: Radical Scavenging Activity
RSE: Radical Scavenging Efficiency
SEM: Scanning Electron Microscopy
TBA: 2-Thiobarbituric acid
TVC: Total Viable Count
USDA: United States Department of Agriculture
XPS: X-ray Photoelectron Spectroscopy

Chapter 1

Introduction

1. INTRODUCTION

Food oxidation is one of the major concerns in food industry processing fatty, oxygen sensitive foodstuffs. It is responsible for the rancid off-flavors and off-odors of food and thus adversely affects consumer acceptance to the food product. Food oxidation occurs through several pathways. Our main concern is auto-oxidation which occurs under mild environmental conditions due to the presence of oxygen and light. Auto-oxidation results in the formation of hydroperoxides which further breakdown to produce aldehydes and ketones that are responsible for food sensory deterioration. Preventing oxidation is achieved through the addition of antioxidants either in the food or the food packaging material. In the present work, the principle of active packaging was used to produce antioxidant films by coating the antioxidant agent (rosemary and clove extracts) on the internal surface of previously treated polyethylene terephthalate (PET) films. Rosemary and clove are known to be potent antioxidants and antimicrobials compared to other natural herbs and they are widely used in food and medicinal applications. Coating of these extracts on the film is achieved by first surface treating the films. Surface treatment techniques include: corona, chemical modification and plasma treatment. The film used in the present work was PET which is known for its good barrier and mechanical properties. Thus, the aim of the present study was 1) to prepare active (antioxidant) PET films by coating them with rosemary and clove extracts and 2) to evaluate their antioxidant activity in contact with mackerel fish tissues. This work will hopefully introduce a simple, easily prepared and inexpensive tool for preserving food. To our knowledge, the contribution of surface treated films in food chemistry is at the early stages of investigation. Hopefully,

this innovative work using coated surface treated films will contribute to food preservation against oxidation.

Framework of the project

PET film → Treatment → (Corona treatment, Chemical modification, Plasma treatment) → Coating → (Rosemary, Clove) → Test for antioxidant activity

DPPH test
Test done in-Vitro between the antioxidant and the DPPH free radical

TBA test
Test done on real fish tissue calculating the amounts of produced secondary oxidation products (malondialdehyde) through their reaction with thiobarbituric acid

13

Chapter 2

Background and literature review

2. Background and literature review

Food packaging has a major role in food preservation through extension of product shelf life, maintaining the quality and safety of processed food and retarding its deterioration. This is achieved by designing a package that functions as a barrier to chemical, biological and physical external factors of food deterioration. Physico-chemical factors include: the effect of gases mainly oxygen, moisture either gained or lost, or the effect of light. Biological factors include microorganisms, insects and rodents. Physical factors also include mechanical damage during handling and distribution through shock and vibration. Another role of packaging is in marketing. This is achieved through innovative package designs and use of appropriate labels that supply information regarding the product especially that on the nutritional value and legal requirements such as use and storage conditions. A third role is in designing a convenient easy-to-use container. For example, a convenient package is a container providing resealability and easy-opening features (Marsh and Bugusu, 2007).

There are several types of food packaging materials; these include metals, paper, paperboard, glass and plastics. Of all the types of packaging materials, plastics are of major importance. This is because they are inexpensive, light, easily shaped, ...etc. There are several types of plastics used in the food industry: polyolefins (e.g. polyethylene, polypropylene), polyamides (e.g. nylon), polyesters (e.g. polyethylene terephthalate), vinyl derivatives (e.g. polyvinyl chloride and polyvinylidene chloride), polycarbonates, etc.

Plastics are of great importance i.e. in milk packaging. For example, high density polyethylene (HDPE) is used to package milk. This plastic type has to be pigmented by titanium dioxide to prevent light transmission and thus protect the milk vitamins from degradation and prevent lipid oxidation. Besides HDPE, PET bottles are also used to package milk. (Robertson, 2013)

Plastics are also used to preserve fermented products such as yoghurt. The most popular type of plastic used for yoghurt packaging is high impact polystyrene (HIPS). Pigments are usually added to the plastic to impart better appearance, light protection and thermoforming properties of PS. HIPS is also used to package cheeses. It is used alone or co-extruded with polyvinylidene chloride (PVdC). Titanium dioxide is used to pigment the package and impart light barrier properties. In addition to this, HDPE or polypropylene (PP) can be used to package cheese.

Bread is usually packaged in low density polyethylene (LDPE) bags sealed with polystyrene (PS) tags. This type of plastic is an excellent barrier to water vapor protecting the bread from hardening without the hazard of mold growth. In some types of French and Italian breads, the bread is stored in oriented polypropylene (OPP) or PET micro-perforated bags.

In case of meat and fish, a wide variety of plastics are used. However, due to the susceptible nature of fresh meat or fish they should be either subjected to a certain preservative treatment i.e. ice or the plastic package should be activated by the addition of antioxidants and/or antimicrobials. Meat treatments include vacuum packaging and modified atmosphere packaging. Vacuum packaging is done using ethyl vinyl acetate/polyvinylidene chloride/ethyl vinyl acetate laminates or more simply polyamide/polyethylene laminates. The first of these combinations has very low oxygen permeability (less than 15.5 mL m^{-2} (24 h)$^{-1}$). A subtype of vacuum packaging is vacuum skin packaging. This type involves the use of films that shrink at the size of meat after vacuum treatment. Modified atmospheric packaging is a method of flushing meat before packaging with gases like nitrogen and/or carbon dioxide. The use of carbon dioxide hinders microbial growth, however causes meat discoloration. Low oxygen treatment maintains the color of meat but it does not have the same ability to protect meat

from oxidation and microbial growth like other gases. (Zhou et al, (2010)) The films used should have good barrier properties to moisture and gases to maintain modified atmospheric packaging as long as possible. The use of several methods of preservation can also be used to ensure better meat or fish preservation (Arvanitoyannis et al. 2012; Sampels, 2015).

The present work focuses on polyethylene terephthalate films and the packaging of mackerel fish.

2.1. Active packaging

Traditional methods for preserving foods entailed the addition of active substances (antioxidants, antimicrobials,…etc.) into the matrix of the food which is subsequently packaged. Recently with the emerging of the idea of active packaging, the active substance is added into the matrix of the polymer or coated on the polymer surface where it is either bound or it migrates into the food during the shelf life of the product. This prevents the active substance from getting consumed immediately after its addition and maintains the quality and safety of the food during storage. Active substances may be synthetic such as Butylated Hydroxy Toluene (BHT) or natural such as natural extracts of thyme and oregano. It is to be noted that the idea of active packaging was first launched in Iceland in the 70's by Dr. Theodore Labuza based on previous work related to this topic. The first innovations came in the form of a wrapper impregnated with sorbic acid to protect cheese from fungal growth. They also incorporated sodium carbonate in specific food packages to remove oxygen. These all did not spark the idea of active packaging until 1976 (in Japan) when a package was invented including iron powder to remove oxygen within the package. This application was widely spread on the market.

17

The most widely used active substances are antimicrobials and antioxidants. There are numerous published articles related to antioxidant and antimicrobial films. In a study carried out by Gemili et al. (2009), L-tyrosine and L-ascorbic acid were used to produce antioxidant cellulose acetate cast films. Another study carried out on cellulose acetate by Lopez et al. (2015) reported the use of red onion extract as a natural antioxidant. In another study, Lopez et al. (2011) added green tea extract in ethylene vinyl alcohol copolymer extruded films to produce an antioxidant package for all types of foods, both aqueous and fatty foods. The same authors also immobilized green tea extract on polypropylene films which provided antioxidant activity when studied in contact with food simulants.

Byun et al. (2010) utilized polylactide films (PLA) incorporating butylated hydroxyl toluene (BHT) and alpha-tocopherol and compared it against polylactide films containing only BHT. It was found that PLA films with alpha-tocopherol and BHT have a higher antioxidant activity than the ones with BHT only. They also found that films containing alpha-tocopherol have higher oxygen permeability and lower water vapor permeability than those with no alpha-tocopherol. The only drawback was that the former had a higher haze than the control film with an increase of about 20%. In addition, a study carried out by Siripatrawan et al. (2010) on green tea extract revealed the possibility of its use in combination with chitosan as antioxidants. Park et al. (2012) incorporated several antioxidants such as carvacrol and eugenol in corn-zein laminated linear low density polyethylene (LLDPE) film. It was found that these films had strong antioxidant activity in beef meat. The films without antioxidants had higher water vapor permeability which was reduced by the addition of antioxidants.

Jouki et al. (2013) used thyme incorporated in quince seed mucilage films. Thyme was incorporated in concentrations ranging from 0 to 2%. The authors found that thyme in

concentration of 1% had considerable antioxidant activity. The films also showed antimicrobial activity against 11 types of microorganisms including *Listeria monocytogenes*. Also, Wang et al. (2014) used *Lycium barbarum* in chitosan films which not only increased the antioxidant activity but also decreased the water vapor permeability of chitosan films. Finally, Ramos et al. (2014) used carvacrol and thymol as potent antioxidants incorporated in polypropylene films.

Regarding antimicrobial active films, Han et al. (1996) incorporated potassium sorbate powder in an extruded LDPE film to control microbial spoilage in cheeses, while Mauriello et al. (2005) prepared a nisin activated LDPE film for the preservation of milk.

2.2.   PET

PET is a polyester produced from a condensation polymerization reaction between ethylene glycol and dimethyl terephthalate (trans-esterification process) or terephthalic acid (esterification process). Ethylene glycol is an ethylene derived colorless liquid and terephthalic acid is a xylene derived crystalline solid. When heated together (in the presence of catalysts), the hydroxyl groups in ethylene glycol react with the carboxyl groups in terephthalic acid forming the polyester and giving water as a by-product (Fig. 1). The presence of several aromatic rings in the PET structure gives the polymer its strength and stiffness (Robertson, 2013).
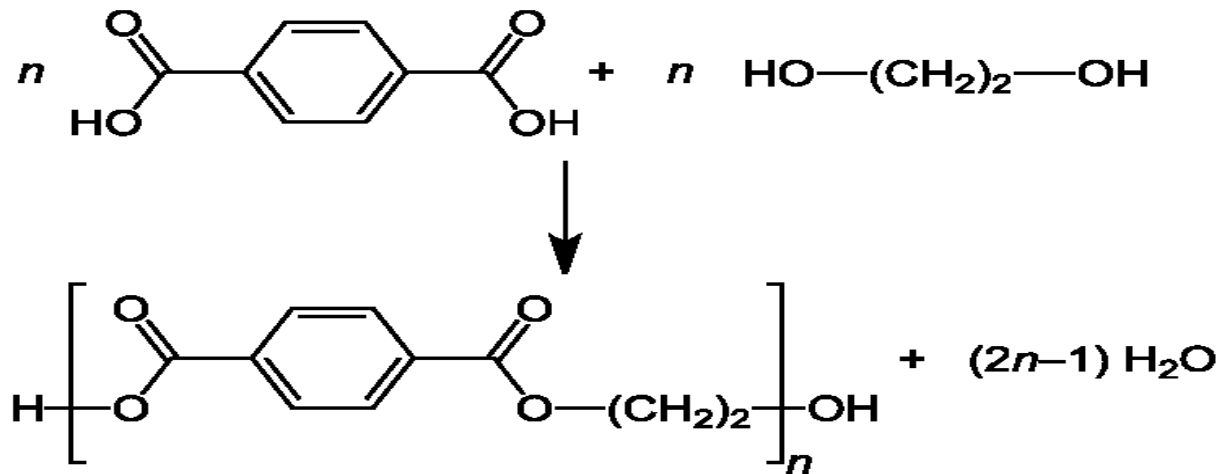
Fig. (1). Synthesis of PET (Kopnik et al, 2005)

PET is known to be a non-reactive, strong, light-weight, and relatively inexpensive polymer. Regarding its safety, PET is considered as safe by major international health agencies, e.g. food and drug administration (FDA) and EFSA (European Food Safety Authority). It does not contain any bisphenol-A (compound used in baby bottles and sports drinks bottles) or phthalates (plasticizers used to soften many plastics). Such compounds are known to be endocrine disruptors. It also does not contain any dioxins which are compounds formed during the manufacture of plastics at high temperatures in the presence of chlorine (vinyl plastics). In case of PET, chlorine is neither used nor formed during its production. According to the society of plastics industry, PET ranks number one regarding safety of plastics compared to 4 for LDPE and 6 for Polystyrene. PET has many applications in several industries. It is used for the packaging of mineral water, fizzy drinks, beer, some shampoos and mouthwash products. It is also used in roasting bags and in the manufacture of food trays. In addition to this, it is used in making clothes and carpet fibers (Ref: PETRA – PET resin association).

20

Moreover, PET can be easily recycled and reused. It is the most widely recycled plastic in the U.S.A. and its recycling can be carried several times. Recycling is achieved by chemical treatment of the polymer to convert PET into its original raw materials (de-polymerization) or by special washing processes to reclaim the clean plastic. The recycled PET can then be used in food packaging, fabrics, automobiles, …etc. In the U.S.A, PET wastes constitute approximately 1% of the landfill space (Ref: PETRA – PET resin association).

There are many published articles that discuss the use of PET in food packaging. Fan et al. (2000) investigated the effect of the stretch ratio in the machine direction and transverse direction on the gas permeability of the film. It was found that stretching in both directions caused the formation of a film of much lower permeability and very high crystallinity compared to the film without stretching. Ke and Yongping (2005) prepared a PET film with the inclusion of organic clay into PET resin. The organic clay was added at concentrations ranging from 1% to 4%. Oxygen barrier properties were then studied. It was found that at a clay concentration of 3%, the oxygen permeability property decreased to its half but at 4% clay concentration, the film could not be prepared. Transmission electron microscopy showed a regular distribution of clay into PET. In another study, Indest et al. (2008) prepared hydrolyzed PET foil by immersing it in 4 M NaOH and 1 M HCl then dissolving in 1,1,2,2, tetrachloroethane and heating. The PET was completely dissolved and the solution was spread over silica quartz crystals. Chitosan was then used to coat the prepared PET film. The effect of pH on the adhesion of chitosan to the PET at equilibrium was then studied. It was found that by increasing the solution pH, the adherence of chitosan to PET decreased due to deprotonation and weakening of chitosan/polymer bonds. Another studied parameter was the ionic strength of a NaOH solution on the adherence of chitosan layer to the polymer at

equilibrium. It was found that the adherence of chitosan increased when 1) the ionic strength of NaOH increased, and 2) the concentration of chitosan solution increased.

Galdi et al. (2008) used a commercial oxygen scavenger (proprietary) extruded along with PET resin to produce antioxidant films. Its addition did not cause any changes or degradation in the polymer matrix. This finding was proven by intrinsic viscosity measurements that are directly related to the molecular weight of the mixture. Differential scanning colorimetry (DSC) measurements were also carried out on PET film *perse* and the PET-oxygen scavenger film, and no change in the crystallinity or polymer thermal behavior was observed. Films containing the commercial scavenger showed a significant decrease in the oxygen permeability as well.

Carneiro-da-Cunha et al. (2010) prepared an aminolyzed (treated with hexanediamine/propanol) charged PET film for layer by layer deposition of sodium alginate and chitosan. Sodium alginate was used because of its ability to remove radioactive toxins as iodine-131. It can also be used as a food thickener for aroma encapsulation and extension of the shelf life of fruit. Chitosan is used as an antibacterial agent. The prepared film showed a decrease in water vapor permeability. By measuring the water contact angle, it was shown that aminolyzing PET decreased its contact angle. However, alginate layer increased the contact angle while chitosan layer decreased it. Also, Yangchuan et al. (2010) incorporated silicon into a PET polymer film. Water contact angle was measured and the film exhibited a very high hydrophobicity. This is important in preparing films with good water barrier properties. It was also found that varying the size of incorporated silicon affected the degree of hydrophobicity.

Contini et al. (2012) used each of alpha-tocopherol and citrus extract sprayed onto PET to develop antioxidant activity for the preservation of cooked turkey meat. The results of the

TBA test showed a two-fold decrease in the degree of oxidation of turkey meat compared to the control film that contained no additives.

In another study, Ghasemi et al. (2012) used nanoclay which was cast extruded along with PET. X-ray diffraction showed that clay nanoparticles were aligned with PET in the machine direction. DSC measurements showed that the film incorporated with clay had higher crystallinity and higher haze. It was also found that the oxygen permeability of the clay containing films was less by 23% than the control film. Regarding the mechanical properties, clay containing PET had higher tensile strength. Cerisuelo et al. (2014) used several antimicrobials including cinnamon bark, marjoram and carvacrol deposited onto PET film by corona discharge. These extracts showed a remarkable decrease in the oxygen permeability compared to that of the control film with no added extracts. The treated film also showed tensile strength and Young's modulus values comparable to that of the control film.

Several studies were carried out to investigate the permeation properties of PET (Ewender et al., 2014). For example, Theodorou and Paik (1992) failed to determine the permeation rates of several aroma compounds through a 16.5 µm PET, since amounts of aroma compounds were below the detection limits. Franz et al. (1993) studied the permeation of Limonene through a 14 µm PET film. They found that PET permitted very minute amounts of limonene through it. They also found that the reaction rate did not reach a steady state and they were not able to determine the lag time. However, Sadler et al. (1996) were able to determine the activation energy and diffusion coefficient of benzene through a 12.7 µm PET film because they used high temperatures (50 – 70°C).

Furthermore, Di Maio et al. (2015) used a commercial oxygen scavenger (proprietary) to produce a three layered PET antioxidant film rather than a one layered one. This three layered configuration along with the oxygen scavenger comprised an efficient antioxidant system.

Finally, Cheng et al. (2015) prepared an antibacterial layer by layer PET film. The layer by layer consisted of the cationic poly(diallydimethyl ammonium chloride) and the anionic silver nanoparticles. The film was then characterized by SEM, XPS and water contact angle measurements. SEM images for a single silver layer showed that there is no aggregation of silver on the surface and that silver is spread homogeneously. Furthermore in case of SEM images for the multilayer PET film, the surface roughness clearly increased preserving surface homogeneity. XPS grams showed that silver exists in its metallic state as was evident from the binding energy calculated from the silver curve. Surface wettability was determined by the sessile drop method using a water contact angle device. It was shown that the surface wettability increased abruptly after coating of the hydrophilic silver nanoparticles.

2.3. Surface treatment

Surface treatment is carried out to either modify physico-chemical surface properties of the film for certain applications or to modify its mechanical properties such as manipulating the orientation, crystallization and crosslinking (Abdel-Bary, 2003). Our main concern in the present work was studying the effect of surface treatment of the film on the surface adhesion properties. Surface treatment is primarily done to enhance the wettability of the film surface thus improving the printing process or to enhance the bonding between two individual layers of plastic films. This is particularly due to the low inherent surface energy, inertness and non-

porous structure of plastic films. Classification of surface treatments is as follows: (Abdel-Bary, 2003).
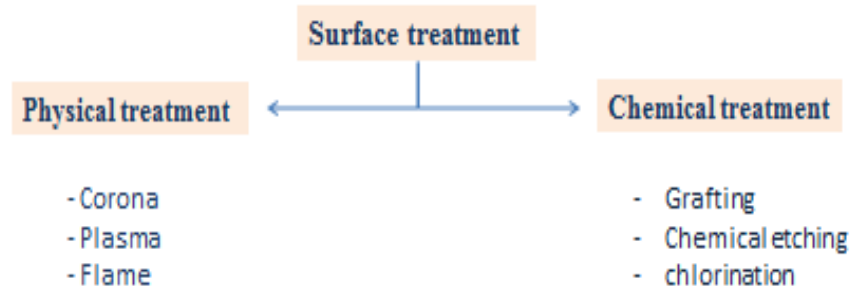
2.3.1. Corona treatment

Corona treatment was first discovered in Denmark by Verner Eisby in 1951 after a request for a safe printing on both sides of a film. Corona treatment occurs via a power supply and a treater station. The power supply provides two electrodes with a single phase high power that ionizes the air in the air gap just above the roll carrying the film. Only the surface placed facing the electrode will be treated (Fig. 2). Both sides of the film can also be treated. The idea behind this type of treatment is that the electrodes with their high power and electric discharge will ionize the air in the air gap through accelerating the electrons present causing the production of an avalanche of electrons at this gap. These electrons will hit the surface of the film with energy that is higher than the energy of the film surface molecular bonding. This will hence cause surface ionization and formation of free radicals that, in turn, will react with oxygen in the gap thus forming functional groups. These groups are responsible for increasing the bonding capability of the surface and thus its wettability. It is to be noted that treatment affects the surface bonds only. Also notable is that treatment and printing should be done at

the time of production. This is the easiest and most economical method of film surface treatment (Sellin & Campos, 2003).
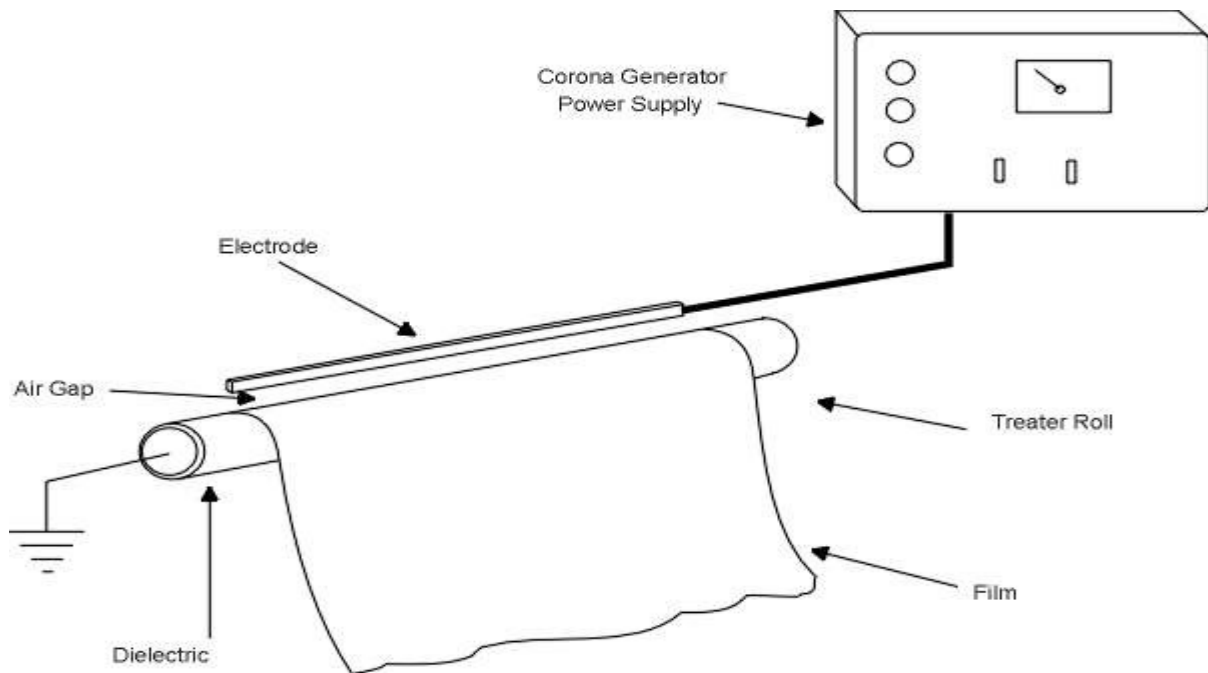
Fig. (2). Corona treatment system (Ref: Plastics consultancy network – Corona Discharge)

A study carried out by O'Hare et al. (2002) investigated the properties of PET after corona treatment. The experiment was conducted under constant power/variable speed and under variable power/ constant speed. It was found that at constant power/ variable speed, the concentration of the active species was independent of speed. However at variable power/ constant speed, the concentration of the active species increased by increasing the power. It was also found that both methods of treatment created the same functional groups. In a study performed by Li et al. (2013) on one side corona treated PET, cellulose nanocrystals were adhered to the treated side. This was attempted to impart anti-fog properties to the film surface, reduce the coefficient of friction of the film and increase the oxygen permeability. Another study by Stepczynska (2013) was carried out to determine the lethal effect of the

corona treatment on microorganisms. The bacterial strains – which are known to cause food contamination e.g. *B.subtilis* and *E.coli* - were inoculated on the surface of polylactide (PLA) food packaging film and the film with these microorganisms was subjected to corona treatment. Bacterial count was determined by microscopic techniques. It was found that this method was effective in killing the bacteria by two orders of magnitude compared to the untreated control film. However, this method was not effective in killing bacterial spores.

2.3.2. Plasma treatment

Plasma is the fourth state of matter. It is an ionized gas made up of positive ions and electrons. These species have high internal energy so that when in contact with the film can cause its activation. They can be generated from a predetermined mixture of gases in a closed chamber under low temperature and pressure (Yousefi et al. 2003). Thus, this method can be used with heat sensitive materials (Fig. 3).
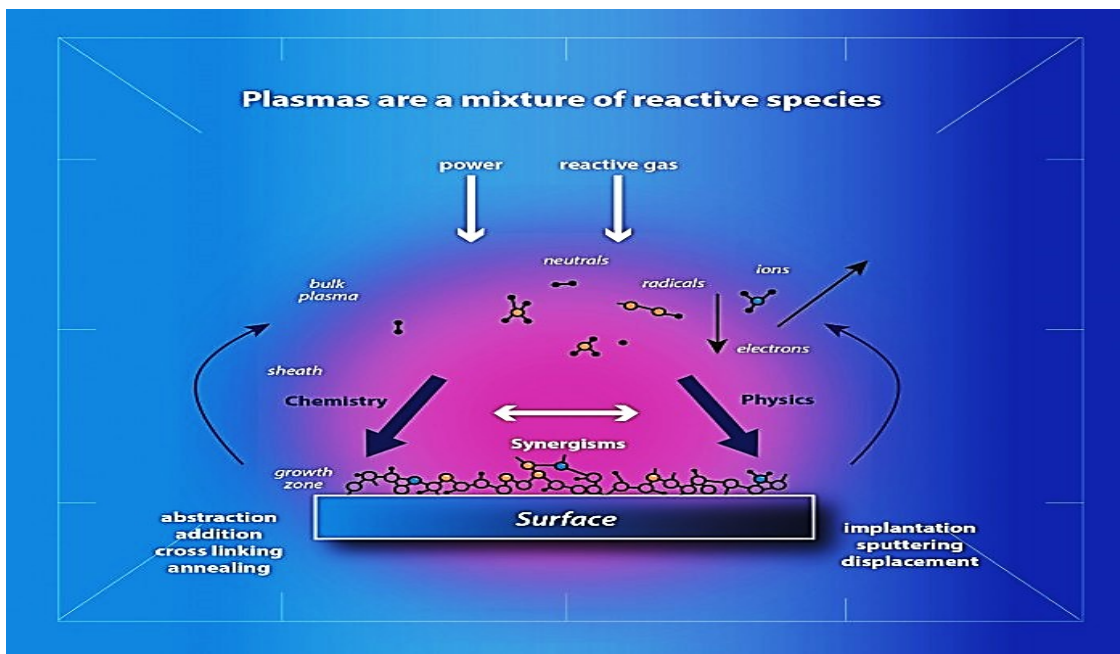
Fig. (3). Mechanism of plasma treatment (Ref.: Henniker plasma: plasma treatment explained in simple terms)

27

A study by Inagaki et al. (1999) investigated the effect of the distance between the argon plasma and the film on surface properties. It was found that at zero distance, a hydrophilic polymer is obtained with surface etching and degradation. On activating the polymer surface at a distance of 80 cm, less hydrophilicity was achieved along with no etching or degradation.

Vartiainen et al. (2005) immobilized chitosan on atmospheric plasma treated biaxially oriented polypropylene film. The film showed a remarkable antimicrobial activity besides its capability to be an excellent oxygen barrier film. This film also showed no migration in food simulants therefore meeting the requirements of the directive 2002/72/EC.

Katsikogianni et al. (2008) studied the effect of plasma treatment on the adherence of *Staphylococcus epidermidis* to a PET film. Plasma treatments were either helium treatment or helium/oxygen mixture treatment. It was found that bacteria preferably adhered and aggregated on the surface of untreated PET rather than the other two treated films. It was also found that bacteria started to increasingly adhere to the plasma treated film on film ageing (when the film was left after treatment for 58 days and measured on intervals within this time period). Also, a study carried out by Asadinezhad et al. (2010) used a physico-chemical approach for adhesion. They initially treated polyvinyl chloride films with coplanar surface barrier discharge plasma then used radical polymerization. Coating with the polysaccharides chitosan and pectin followed. This study was successful in hindering the adhesion of *S.aureus* bacteria by 30% and *E.coli* by 50%, therefore it was concluded that this film could be used in medical applications. Recek et al. (2013) treated PET films either by oxygen plasma to form hydrophilic films or tetrafluoromethane to prepare hydrophobic films. It was found that the amount of protein adsorbed on the hydrophilic film was as twice as that of the hydrophobic films. Therefore the hydrophilic films are better used to study cell adhesion and proliferation.

Lei et al. (2014) investigated the antimicrobial effect of plasma treated polyethylene terephthalate/polypropylene (PET/PP) composite films - dipped in polylysine, potassium sorbate, sodium benzoate or calcium propionate - against *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli.* It was found that the film completely inhibited the latter two species, while its inhibitory effect did not exceed 85% with the former one. It was also found that the release of antimicrobials increased with increasing temperature and acidity of the medium.

2.3.3. Grafting (chemical modification)

It is the process of graft copolymerization of , for example, vinyl monomers onto the surface of the film. This kind of treatment has produced good results but the only drawback is its high cost. This process occurs by one of several methods; chain transfer, redox reaction, photochemical initiation and gamma radiation induced copolymerization (Abdel-Bary, 2003). In the present study, we used films that were treated by a commercial surface film treatment called **'acrylic chemical modification of film surface treatment'.** Acrylic derivatives could be acrylamide, acrylic acid or a salt of acrylic acid. This is a proprietary process for the company. There are numerous articles published on the use of acrylic surface treated films. In a study by Sun et al. (2010), PET was activated by atmospheric pressure plasma at different times to generate free radicals followed by its immersion in an inverse emulsion of acrylic acid for graft polymerization of the acrylic acid. Surface morphology was studied by SEM and showed that surface roughness increased with increasing the time of plasma treatment. Also, contact angle measurements of the grafted polymer surface showed highly increased hydrophilicity. In another study by Ping et al. (2010), it was shown that the activation of PET with gamma irradiation in the presence of acrylic acid monomer solution was possible.

29

Coating of silver nanoparticles then took place to impart antibacterial properties. XPS measurements showed the presence of silver in the metallic state. Furthermore, antibacterial properties were documented by suspending the film in a suspension of *E. coli* viable cells and studying the decrease of these viable cells after 4 hours. The film showed potent antibacterial activity.

2.4. Measuring surface energy

Surface energy of solids is the ***work per unit area*** done by the force that creates the new surface. (Ref: Physics for civil engineering – lecture 8: surface tension and surface energy, by (Peter Eyland) university of New South Wales). In order to measure it, we use liquid droplets spread over the surface to measure contact angle and thus get the surface energy. According to Young's equation (Equation 1), there is a relation between the contact angle θ, the surface tension of the liquid σ_l, the interfacial tension σ_{sl} between liquid and solid and the surface free energy σ_s of the solid (Fig. 4). (Ref.: Kruss – surface free energy)

$$\sigma_s = \sigma_{sl} + \sigma_l \cdot \cos\theta$$

(Equation 1)

(Ref.: Kruss – surface free energy)
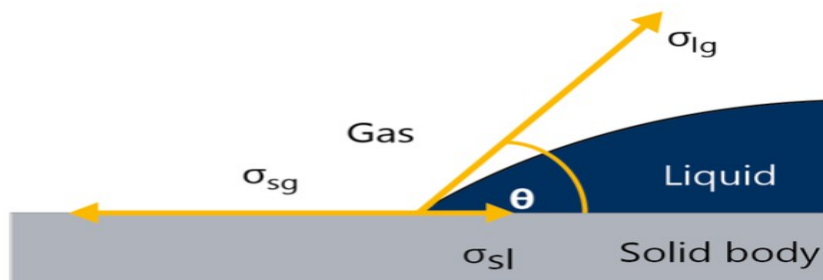
Fig. (4). Water contact angle measurement (Ref.: Kruss – surface free energy)

There are several factors affecting the surface energy of solids. One of these factors is temperature. When the temperature increases, atoms will vibrate and cause a decrease in the cohesive forces between atoms. Thus, when the net inward cohesive force is decreased, surface energy will decrease. Another factor is contamination. Contaminants change the net inward cohesive forces and thus cause a decrease in the surface energy. It is to be noted that the degree of wetting affects the shape of the droplet over the surface (Fig. 5).
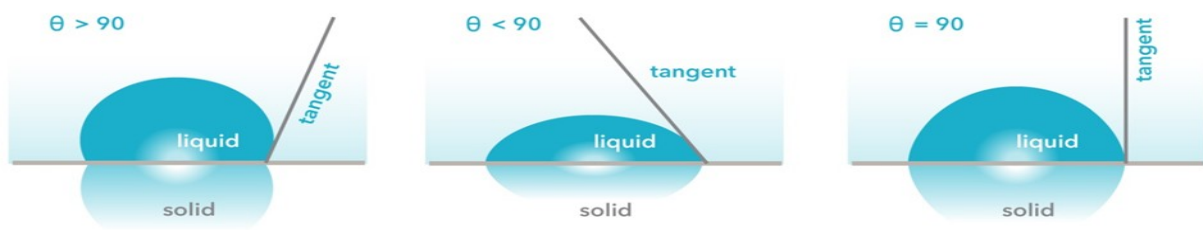
Fig. (5). Phenomenon of wetting at different contact angles (Ref.: Biolin scientific: attension application)

The mechanism of wetting and de-wetting can be explained as illustrated in (Fig. 6). Let F_{SG} the upward force between the solid and the gas, F_{SL} the downward force between the solid and the liquid and F_{LG} the inclined force between the liquid and the gas.
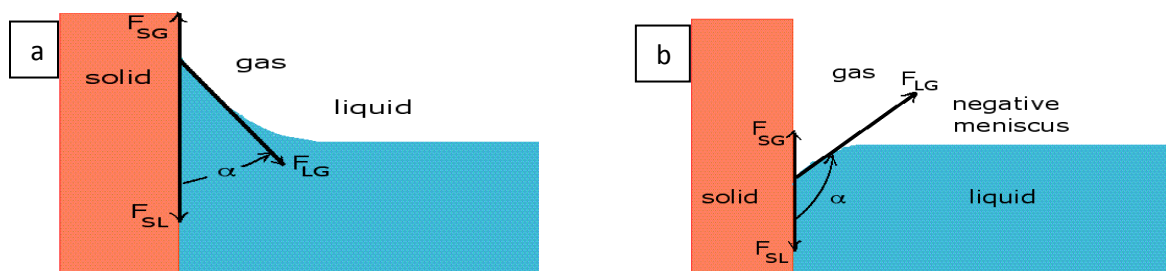
Fig.(6): An illustration of the forces acting in (a) wetting phenomenon; (b) de-wetting phenomenon (Ref: Physics for civil engineering – lecture 8: surface tension and surface energy, by (Peter Eyland) university of New South Wales)

When F_{SL} and F_{LG} are in the same direction as in Fig. (5a), the cosine of angle (a) between them is positive, thus the meniscus is positive and wetting takes place. On the contrary, if the

cosine of the angle between F_{SL} and F_{LG} is negative (Fig. 5b), then the meniscus is negative and no wetting takes place. In these two cases, we assume F_{SG} to be very small compared to the other two forces.

There are several methods for measuring surface energy of plastic films. The first method is **drop shaped analysis.** It involves the study of contact angle between the intersection point of the solid surface and the drop contour. The second method is **top-view distance method**. This method involves measuring the curvature of a drop surface that is related to the contact angle by measuring the distance between the reflected dots of light from the surface of the droplet. The contact angle measured then is converted into surface energy through Equation 1 and Table 1.

Table (1). Values of surface tension corresponding to their contact angles

Water contact angle	Dyne test approximation, Dyne/cm
54	45
55	45
56	45
57	44
58	44
59	44
60	43
61	43
62	43
63	42
64	42
65	42
66	41
67	41
68	41
69	40
70	40
71	40
72	39
73	39
74	38
75	38
76	38
77	37
78	37
79	37
80	36
81	36
82	35
83	35
84	35
85	34
86	34
87	34
88	33
89	33
90	32
81	32
92	32
93	31
94	31
95	31

33

2.5.   Oxidation

Oxidation of food lipids involves the addition of oxygen and subsequent breakdown of lipids. Oxidation occurs usually in the form of **auto-oxidation** of unsaturated fatty acids, **thermal oxidation** of fats and oils, **light induced oxidation** and **enzymatic oxidation.** Auto-oxidation occurs between a) a carbon to carbon double bond and oxygen or b) a carbon atom in alpha position relative to a double bond and oxygen to form free radicals. These radicals initially form hydroperoxides (primary oxidation products) which in turn breakdown to secondary oxidation products (aldehydes, ketones) responsible for the rancid off-flavors and off-odors of food (Choe & Min, 2009). In light induced oxidation the process is initiated by molecules known as 'initiators' i.e. chlorophyll which convert triplet to singlet oxygen. This in turn attacks unsaturated fatty acids and the rest of the oxidation mechanism is the same as that of auto-oxidation. Enzymatic oxidation occurs by specific food enzymes (polyphenoloxidase) on different molecules (mainly aminoacids) that cause browning of food. In the present work, we will focus on lipid auto-oxidation. This occurs through three steps; initiation of free radical formation, propagation with formation of long-chain radicals and termination with formation of non-radical products (Figs.7 and 8). The most important moieties involved in oxidation are the unsaturated moieties; oleic, linoleic and lenolenic which have different rates of oxidation; oleic< linoleic< linolenic.

Steps: initiation:
   $RH \cdots\cdots R\cdot$
Propagation:
$R. + O_2 \cdots\cdots ROO\cdot$
$ROOH \cdots\cdots RO\cdot + OH\cdot$
$ROO\cdot + RH \cdots\cdots ROOH + R\cdot$
Termination:
$R\cdot + R\cdot \cdots\cdots RR$
$RO\cdot + RO\cdot \cdots\cdots ROOR$
$RO\cdot + R\cdot \cdots\cdots ROR$
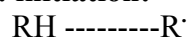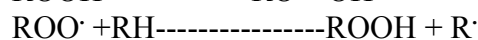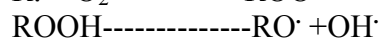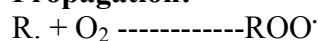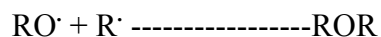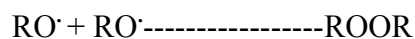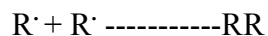
Fig. (7). Auto-oxidation mechanism of unsaturated fatty acids,where RH is the unsaturated fatty acid, R **.** is the free radical and ROOH is a hydroperoxide whose degradation is responsible for the off-odors and flavors (Eskin &Shahidi, 2012)

The scheme in Fig. 7 shows the reaction of hydroperoxides to give aldehydes, ketones, alcohols or hydrocarbons.
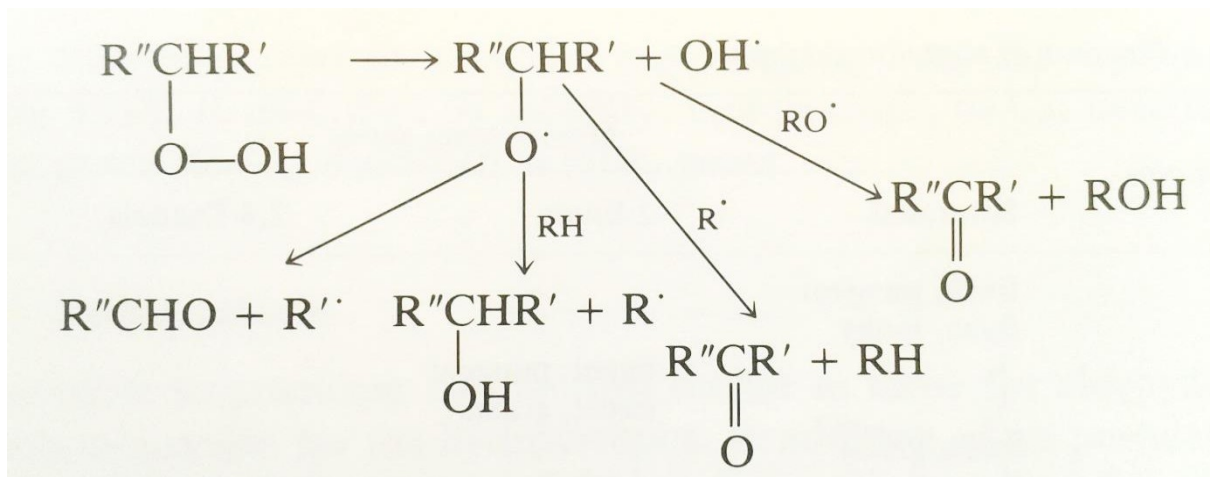
Fig. (8). Mechanism for the formation of aldehydes, ketones, alcohols and hydrocarbons through lipid auto-oxidation (Allen & Hamilton, 1989)

2.6. Antioxidants

They are compounds that inhibit the formation of free radicals or delay the propagation of the peroxidation process. This delaying occurs by several mechanisms. One of these mechanisms is by chelating metal ions that can decompose peroxides. Another mechanism is by decreasing the concentration of localized oxygen and also quenching oxygen radicals that are responsible for peroxide formation. The effectiveness of an antioxidant depends on the activity of the antioxidant and its solubility; i.e. its ability to penetrate food tissues and emulsion interfaces. The effectiveness of the antioxidant is strongly related to the activation energy, rate constants, and oxidation-reduction potential. The reactions of inhibition and chain propagation are exothermic; if their activation energy is high then the effectiveness of the antioxidant will decrease. The most potent antioxidants are those which interrupt the free radical chain

35

reactions. These antioxidants usually have a phenolic ring structure. They perform their antioxidant function by one of two pathways; they either donate a hydrogen atom to the free radical in a process called hydrogen transfer or a process named single electron transfer. In single electron transfer, the antioxidant will either pick up the free radical electron that will be stabilized on the antioxidant's phenolic ring by resonance forming quinone structure and thus form a more stable structure- for both antioxidant or radical- or give a single electron to the radical to form a stable anion with an even number of electrons (Fig. 9). The bond dissociation energy of O-H group is an important factor in these mechanisms (Choe & Min, 2009). The strength of O-H bond depends greatly on the substituents or functional groups on the benzene rings because they are responsible for the stability of the phenolic radical. For example, the presence of methyl, methoxy or tertiary butyl groups in the ortho- position greatly reduces the energy of O-H bond more than their presence in the meta- position. In addition to this, the presence of antioxidants in alkaline medium increases their rate of the reaction more than when they exist in either neutral or acidic media. Natural antioxidants are usually present in plant tissues for their protection from various internal and external factors. Such antioxidants include phenolic acids, carotenoids, flavonoids and tocopherols (Choe & Min, 2009). Phenolic acids are closely related to flavonoids. Flavonoids have hydroxyl groups or sometimes characterized by a catechol group in the B ring (Fig. 10). One of the drawbacks of phenolic acids is that they cannot be used with oils due to poor solubility in lipids. Unlike phenolic acids, carotenoids are used mainly with fats and oils, for example palm oil is a rich source of carotenoids. Another drawback of phenolic acids is that they are only used at alkaline pH. At high pH, they are converted to the phenolate form that has a higher and more rapid radical scavenging activity. Tocopherols have a higher antioxidant activity against alkyl peroxy radicals compared to alkyl radicals. This is because the difference in reduction potential

36

between tocopherols and the former is greater than that of tocopherol and the latter (Choe & Min, 2009).
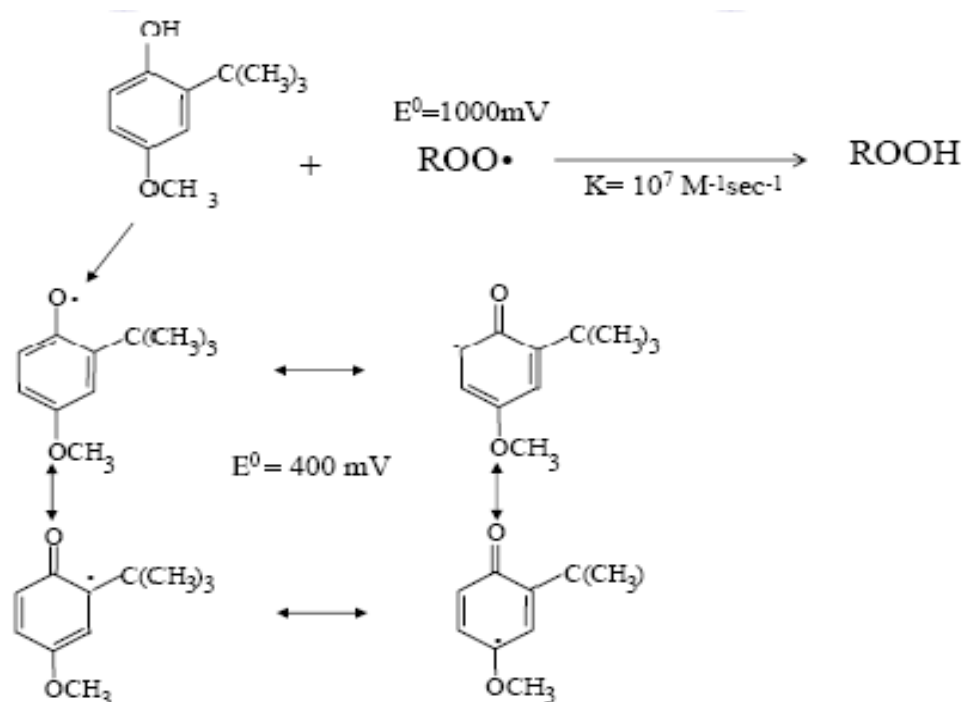
Fig. (9). Resonance stabilization of antioxidant radicals (Ref: Iastate university – lipid oxidation)
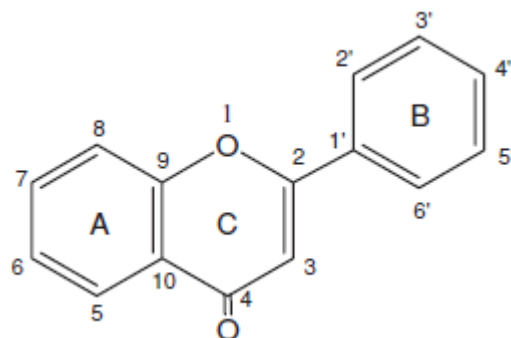
Fig. (10). Structure of flavonoid back bone

2.7. Chemical composition of natural antioxidants

2.7.1. Rosemary

Rosemarinus officinalis or rosemary is an evergreen shrub 50 to 150 cm high, made up of brown branches. The herb has been used by ancient Egyptians, Mesopotamians, Chinese, Greeks and Indians in culinary, medicinal and cosmetic applications. The medicinal parts of the plants are oil extracted from the dried or fresh leaves or from the flowering twigs. This plant has mild antimicrobial and antiviral properties. Anti-listerial properties were found in rosemary polyphenol extract with a minimum inhibitory concentration of 0.083 mg/mL (Bubonja-Sonje et al., 2011). It has also been used in dyspeptic ulcers, hypotonic circulatory disorders and rheumatic disorders. Besides, it has been used to treat oligomenorrhea, amenorrhea and dysmenorrhea. It has also some dermatological uses in eczema, and the healing of wounds. It has been used topically for myalgia, sciatica and intercostal neuralgia. According to the European food safety authority (EFSA), the use of rosemary does not pose any health concerns. According to numerous studies, rosemary does not pose any genotoxic side effects (EFSA, 2008). It was concluded that rosemary has very low acute and sub-chronic toxic effects. It was also found that at high doses, liver enlargement takes place without increase in the enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Furthermore, it was suggested that this enlargement is not considered a toxic effect but rather an adaptive action to the high doses of rosemary intake (EFSA, 2008). Another study on the hepato-protective activity of rosemary on rats was performed by Raskovic et al. (2014). They induced liver injury in rats by carbon tetrachloride then treated the rats with 5 and 10 mg/kg rosemary essential oil. They found that rosemary has the ability to prevent the lipid peroxidation of liver homogenates induced by carbon tetrachloride. Studies have also shown that rosemary exhibits anti-cancer activity in rats.

Comparing rosemary to a mixture of butylated hydroxyl anisole (BHA)/butylated hydroxyl toluene (BHT), it was found that rosemary was able to preserve the color of pork sausage. It was also found that the ability of rosemary at concentrations of 2500 ppm in preserving refrigerated fresh pork sausage and cooked-frozen pork sausage was comparable to the ability of BHA/BHT at its highest permissible concentration, but was superior to BHA/BHT in raw-frozen pork sausage (Sebranek et al., 2005).

BHA and BHT are synthetic antioxidants prepared in the laboratory. BHA is a mixture of two isomers; 2-tertiary BHA and 3-tertiary BHA where the 3-tertiary isomer is the most widely used commercially. It is noted that BHA is more frequently used than BHT because it can withstand higher temperatures thus, it can be used in baking. Both antioxidants are mainly used in fatty foods. They also have phenolic odors which may affect the odor of the food. The main problem with BHA and BHT is that they pose health risks. They have been shown to be carcinogenic and mutagenic (Shahidi and Zhong, 2005). They are not metabolizable by some people which may cause behavioral changes. Health risks also include: asthma, allergy, attention deficit hyperactivity disorder (ADHD), weight gain, vasculitis, dermatitis, rhinitis, eye problems and headache. According to the code of federal regulations No. 21 of FDA, BHA and BHT can be used in food within the limits set by the FDA for each type of food. Any food that contains BHA or BHT has to be labeled for containing any of these synthetic antioxidants including their concentration. Their concentration should never exceed 0.02% and they cannot be used in fish. According to the USDA, their weight should not exceed 0.01% of the weight of fat. In Japan, BHA is approved to be used at a concentration of up to 0.5-0.7% of the acceptable daily intake. Both BHA and BHT are approved to be used in the U.S.A., Canada and Europe (Shahidi and Zhong, 2005). Unlike BHA and BHT, rosemary has been

classified by FDA and the European Union as "generally recognized as safe" and thus can be used in food preservation. Rosemary extracts have been obtained by several methods. By comparing the extraction power of conventional solvents such as water, methanol and ethanol, ethanolic extracts proved to be the best yielding about 18% w/w (weight of extract/weight of plant tissue) which exceeds the yields of water and methanol extracts of 13% w/w and 4%w/w, respectively(Tariq et al., 2013 - Tavassoli & Djomeh, 2011).

Rosemary extract has three main components; carnosic acid, carnosol and rosmarinic acid (Table 2). In a study carried out by Jordan et al. (2012), the methanolic extract of rosemary showed significant antioxidant activity due to the presence of rosmarinic acid. On selecting 27 methanolic extracts from 150 plants of different ratios of carnosic acids and carnosol and testing them, it was found that antioxidant activity is mainly affected by carnosol rather than carnosic acid. In addition to this, it was found that carnosol plays an important role in the antibacterial activity of the extract especially against *Staphylococcus aureus* and *Listeria monocytogenes*. According to the publisher, this finding contradicts several publications in the literature which showed that carnosic acid exhibited higher antibacterial activity than carnosol.

The rosemary plant in the Mediterranean basin is exposed to an irregular supply of water and nutrients and thus suffers oxidative stress. This causes the plant to produce the compound carnosic acid and other antioxidants to protect the plant from oxidative stress. This compound scavenges reactive oxygen species giving o-methylated diterpenes. Such diterpenes have high lipophilicity which permits their binding to phospholipids of the plasma membrane and thus reinforces it against reactive oxygen species ((Birtic et al., 2015).

Table (2). HPLC fingerprints for rosemary dried leaves, and extracts D74, F62, ARD, AR and RES (EFSA, 2008)

Parameter Unit	Dried Leaves	Extract D74	Extract F62	Extract AR	Extract ARD	Extract RES
Phenolic diterpenes Carnosic acid mg/g Carnosol mg/g	15-25 1-2	240-260 35-45	155-175 15-17	30-50 20-30	60-80 18-22	110-150 30-50
Triterpenic acids Betulinic acid mg/g Sum oleanic + ursolic acid mg/g	10-15 20-35	130-145 130-150	85-110 185-220	15-30 35-45	55-65 130-170	60-80 90-120

D74= extraction with supercritical CO2   **ARD**= extraction with ethanol and deodorized by distillation
F62= extraction with acetone   **RES**= extraction with hexane and ethanol
AR= extraction with ethanol

There are several published papers on the use of rosemary in food packaging films. Perone et al. (2011) incorporated rosemary oil in hydroxyl propyl methyl cellulose (HPMC) films and found that rosemary decreased the water vapor permeability of the films. Abdollahi et al. (2012) incorporated rosemary essential oil in chitosan films. They found that rosemary increased the antibacterial property and total phenol content of chitosan films. They also found that rosemary enhanced the transparency of neat chitosan films. Furthermore, Barbosa-Pereira et al. (2014) formulated LDPE films coated with rosemary. They measured the TBA antioxidant activity test of the films and found that rosemary reduced the degree of oxidation of beef by about 49.3% by the 17th day compared to the control.

2.7.2. Clove

It is the flower bud of the plant *Eugenia caryophyllata* found in Indonesia, India, Tanzania and Madagascar. It has a nail-shaped appearance and has been used since ancient times in culinary practices. Clove has been mainly used in dentistry, headaches, nausea and digestive disorders. The main active ingredient of clove is eugenol constituting about 85 – 95% of clove (Table 3).

Eugenol is a phenyl propanoid that is weakly acidic, slightly soluble in water and soluble in organic solvents. Clove has many pharmacological and medicinal uses. One use is through its antibacterial properties. It forms a clear inhibition zone against several bacteria; *B.cereus, E.coli, S.aureus* and *Helicobacter pylori.* It was found that clove along with vancomycin have a synergistic effect in penetrating the cell wall of gram negative bacteria at concentrations of 1 mM. Also, clove proved to have antifungal activity where it shows a synergistic activity with fluconazole or amphotericin B against *Candida albicans* (Khan et al., 2012). Due to its properties, clove is considered as a natural preservative for food, alternative to synthetic preservatives that may cause adverse health effects. In a study by Pandey et al. (2011), it was found that the methanolic and ethanolic extracts of clove show a significant antimicrobial activity against gram positive and gram negative bacteria when compared to the synthetic antibiotic tetracycline. Both showed the highest activity against *Pseudomonas aerugenosa.* Regarding the safety of clove, there is no apparent side effect to this spice other than that it hinders blood clotting. This finding is important in case of patients who have bleeding disorders. Also, people subjected to surgery may suffer from bleeding and therefore they have to stop taking any clove two weeks before surgery. Furthermore, clove may be unsafe in case of children as it may cause liver disorders, seizures or fluid disturbances (Lane et al., 1991).

The chemical composition of clove as determined by Gas chromatographic/mass spectrometric analysis is shown in Table 3.

Table (3). Chemical composition of clove oil (Alma et al., 2007)

Compounds	RI	%
2-Heptanone	889	0.04
α-Pinene	921	0.01
p-Cymene	1023	Tr*
Limonene+1,8 Cineole	1029	0.01
2-Heptyl acetate	1046	0.04
(E)-β-Ocimene	1051	0.33
2-Nonanone	1092	0.02
Linalool	1098	0.01
Methyl salicylate	1189	0.07
p-Allyl phenol	1260	0.19
Eugenol	1361	87.00
α-Copaene	1372	0.10
β-Caryophyllene	1412	3.56
α-Humulene	1446	0.40
Δ-Cadinene	1518	0.04
Eugenyl acetate	1526	8.01
Caryophyllene oxide	1573	0.10
2(12),6(13)-Caryophyllen-dien-5-ol	1627	0.02

*RT= retention index

There are many published papers on the use of clove in packaging films. Arancibia et al. (2013) designed a soy protein-lignin insecticidal package containing 3% clove oil. They tested this film against *Ceratitis capitata* fruit fly and found that 40% of the flies were knocked down after 4h and that 90% of the flies died after 20h. Muppalla et al. (2014) prepared a carboxymethyl cellulose/polyvinyl alcohol/clove film by the film casting method. They found that clove containing films had antimicrobial activity and were able to extend the shelf life of meat for 12 days compared to the control film that was kept only for 4 days. Also, Wu et al.

(2014) prepared polylactide films with the addition of clove. They found that clove containing films had a higher water vapor permeability and a greater antioxidant activity than the control films.

2.8. Methods for the determination of antioxidant activity

2.8.1. DPPH method

2,2- Diphenyl-1-picryl-hydrazyl (DPPH) is a free radical molecule whose free electron is stabilized by resonance. This molecule in solution shows a strong purple color with an absorption maximum at 517 nm (there is variation in the literature in the wavelength used, being between 517 and 528 nm). When this molecule comes in contact with an antioxidant, DPPH gains a hydrogen atom from this free radical scavenging antioxidant and the solution turns yellow in color (Fig. 11). The change in color is stoichiometric to the number of free electrons being captured.
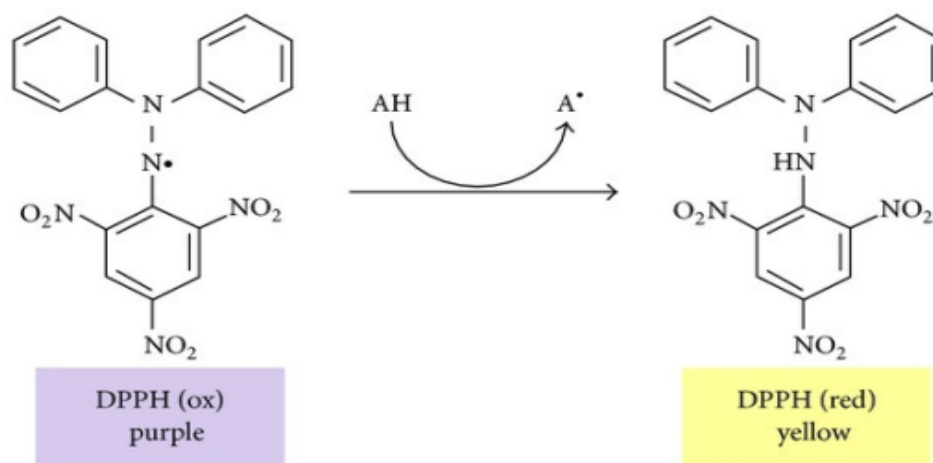
Fig. (11). Reaction of antioxidant with DPPH (Teixeira et al., 2013)

Antioxidants being studied may be either water soluble, fat soluble, insoluble or bound to cell walls. This will affect the rate of the reaction since DPPH is not water soluble as well as the method of extraction. Some reactions are fast ending at 5 minutes, others are intermediate

44

taking from 5 to 30 minutes and others are slow taking hours to complete. It is to be noted that the literature mostly reports 20 to 30 minutes but it is best preferred that the reaction is continued until completion since the rate of reaction differs among substrates. The reaction is known to be complete when readings reach a plateau. This means that the difference between two consecutive readings is not more than 5%. It is to be noted that for an antioxidant to be tested for its activity it has to be completely dissolved in the chosen solvent (Karadag et al., 2009).

Results are plotted in several ways. One way is to plot % reduction of DPPH against concentration to get the slope. From the slope, IC_{50} is calculated. IC_{50} is the concentration of antioxidant required to decrease the concentration of DPPH by 50% (Kedare et al. 2011). This value is inversely proportional to the strength of the antioxidant. Another parameter that is used to express degree of antioxidant activity is Radical Scavenging Efficiency (RSE). This parameter value is calculated as the ratio between the initial scavenging rate at the first minute and IC_{50}. The drawback of this value is that it is dependent on the initial DPPH radical concentration (De Beer et al., 2003).

This method is advantageous in being simple and inexpensive. In order to perform the test, one needs only a UV-visible spectrophotometer. However, this method has also drawbacks. One of these drawbacks is that DPPH is light, solvent and oxygen sensitive which may affect the absorbance value. Another drawback is that the reaction kinetics of antioxidants with DPPH may not be the same as their reaction *in vivo*. Also some antioxidants have absorbance values that overlap with that of DPPH, e.g. carotenoids. A fourth drawback is that sometimes the reaction between DPPH and antioxidants is not linear which makes it difficult to plot a curve and calculate IC_{50}. Also, the presence of any impurities either acidic or basic may affect

the ionization equilibrium of phenolic antioxidants and thus affect the parameters measured. (Karadag et al., 2009).

2.8.2. The oxygen radical absorbance capacity (ORAC)

This method is based on 2, 2′-azobis (2-amidinepropane) dihydrochloride (AAPH) to produce hydroxyl radicals that react with fluorescein to obtain a non-fluorescent compound (Fig. 12). Antioxidants added delay this reaction and its activity is determined by measuring the area under the fluorescence decaying reaction curve and lag times. This reaction is a simulation to the human cell degeneration within the human body. This test can be used in food and cosmetic applications (Bentayeb et al., 2014).
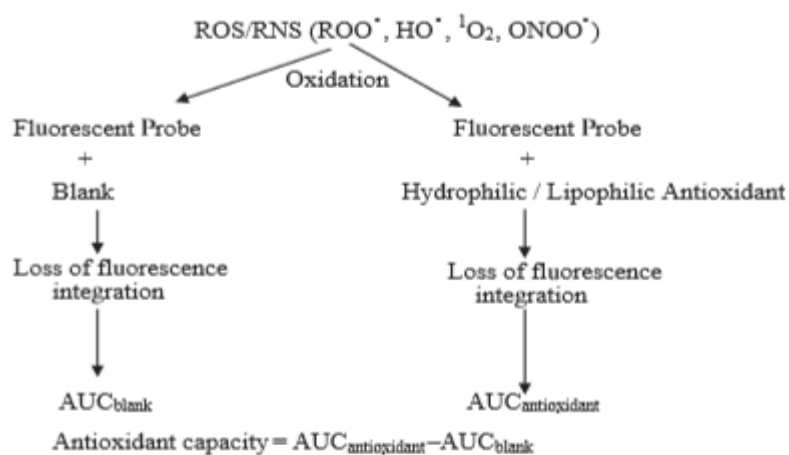
Fig.(12). Mechanism of ORAC test, AUC stands for Area UnderThe Curve. (Singh & Singh, 2008)

2.8.3. The ferric reducing ability of plasma (FRAP) method

This method depends on the ability of antioxidants to reduce ferric tripyridyltriazine to its ferrous form at low pH and at wavelength of 593 nm. The reduced form exhibits an intense blue color. This method is easy, automated and gives a linear response over a wide range of

46

concentrations. However, it is non-specific and any substance that has a lower positive redox potential than the ferric ion will cause its reduction (Benzie and Strain, 1996).

2.8.4. Thiobarbituric acid (TBA) test

Studying lipid peroxidation is very important as this is responsible for food rancidity. Besides, lipid oxidation products are partly responsible for heart related diseases, cancer and aging. One of the degradation products of lipid peroxides is malondialdehyde (MDA). Studying the extent of lipid peroxidation through MDA is carried out by the TBA test. This test can be performed by several means, either heating the sample with TBA and separating the pink complex, or distilling the sample and reacting it with TBA, or extracting the lipid portion with organic solvents and then reacting with TBA, or by extracting MDA with trichloroacetic acid or perchloric acid and then reacting with TBA. Actually distillation of the sample is the most widely used method. This method has the drawback that heating the sample may result in further degradation of other lipid peroxides to form additional MDA and hence yield higher TBA values. In all cases, the resultant of the test is a pink complex whose absorbance is measured at 532 nm. The TBA test is generally considered as an insensitive and non-specific method. The reaction involves two molecules of the chromogen TBA reacting with MDA (Fig. 13) (Botsoglou et al., 1994).
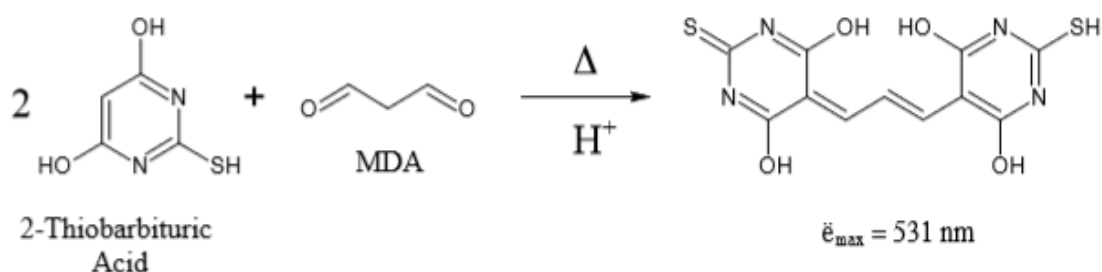
Fig. (13). Reaction of 2-thiobarbituric acid with malondialdehyde (Ref.: Eagle biosciences: TBARS ELISA)

47

Chapter 3
Materials and methods

3. Materials and methods

3.1. Materials

Corona treated and chemically modified films were supplied by the Egyptian packaging industry. Plasma treated films were prepared in Greece using a Plasma surface Tech, model: ATTO lab scale plasma treater operating with oxygen at an oxygen pressure of 0.6 mbar, and a power output of 50 W. Plasma treating time was 100 seconds.

Absolute ethanol used in the preparation of coated films was purchased from Fischer Company (UK). All solvents were of analytical grade.

Rosemary and clove were bought from the Egyptian market. 1,1-diphenyl-2-picrylhydrazyl (DPPH), BHT and TBA were purchased from Sigma Aldrich (Greece). Silicon oil was purchased from Merck-Schuchardet (Greece).

Mackerel fish was purchased frozen from the Egyptian market.

3.2. Film preparation

A mass of 0.0701 g of semi-dried rosemary leaves or clove flowering buds were placed in paper thimbles which were transferred to the extractor of a Soxhlet apparatus. A volume of 80 mL of absolute ethanol was placed in the round bottom flask of the apparatus and extraction was carried out for four hours in case of rosemary and 2.5 hours in case of clove. Then the prepared extract was poured on the surface of the PET film which was fixed to the edges of a 20x20 cm

Soxhlet apparatus

glass mold. The set up was left under the hood for 24 h to evaporate the ethanol.
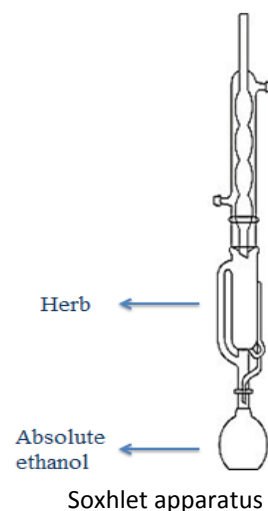
3.3. Determination of the antioxidant activity of the films using the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) test

This test was performed by dissolving 0.0040 g of DPPH powder in a 100 mL volumetric flask. DPPH was dissolved in small amounts of absolute ethanol step by step until complete dissolution (at 40 ppm concentration). The flask was placed in the fridge for 2 hours to stabilize.

Films were cut into small pieces (1x1 cm) and extracted in a beaker containing a small volume of absolute ethanol. The beaker was placed on a magnetic stirrer for complete extraction. The extract was added to a 50 mL volumetric flask then another portion of ethanol was added in the beaker - to ensure total extraction of the antioxidants - and stirred. The procedure was repeated until filling the volumetric flask to the mark. Aliquots of 8, 6, 2, 1 mL from this flask were transferred into 10 mL volumetric flasks, complete to the mark with ethanol and shaken.

A volume of 0.1 mL from every flask was added to 2 mL DPPH stock solution in a cuvette and the absorbance was measured at 517 nm on Amersham Biosciences Ultrospec 3100 pro spectrophotometer.

Absorbance values were substituted in the following equations (Brand-Williams et al. 1995), (Anagnostopoulou et al. 2006):

%Reduction in DPPH absorbance = $(A_0 - A_t) *100/A_0$ …………..Equation (2)

This value is also called radical scavenging activity (RSA) where A_0 is the initial absorbance of DPPH without antioxidants and A_t is the absorbance of DPPH after addition of the antioxidant. (Brand-Williams et al. 1995), (Anagnostopoulou et al. 2006):

%DPPH remaining = A_t*100/A_0 ……………………………….Equation (3)

This value (%DPPH remaining) is plotted against the concentration of the extract to yield a straight line from the equation of which the IC_{50} is calculated (IC_{50} is the concentration (expressed as mg/ml) of the antioxidant at which half of the radicals are scavenged) Then the antiradical efficiency (AE) is calculated as follows: (Brand-Williams et al. 1995), (Anagnostopoulou et al. 2006):

$AE = 1/IC_{50}$ …………………….. …………………………………. Equation (4)

This value is used to facilitate expressing antioxidant activity as it is directly proportional to the antioxidant activity unlike IC_{50}.

3.4. Total viable count (TVC)

Ten grams of minced fish were added to 90 mL of 0.1% peptone water and homogenized using a model polytron PT-10-35-GT mechanical homogenizer presterilized using alcohol. Several dilutions were prepared from this stock solution and inoculated on nutrient agar petri dishes. The petri dishes were then incubated at $30^{\circ}C$ for 48 hours. The resultant colonies after incubation were counted manually. This test is performed to determine when the samples will become microbiologically spoiled and thus TBA test should be discontinued. Two replicates were performed for each sample.

3.5. Determination of antioxidant activity using Thiobarbituric acid (TBA) test
a- Fish preparation

The mackerel fish was cut open to remove the skin, viscera, head and bones. The fish tissue was then minced in an electric mincer. Ten grams of minced fish were then sandwiched between two treated/coated films (10x10 cm), sealed using a thermal sealer. Bags were then stored in the fridge.

b- Thiobarbituric acid test

A volume of 97.5 mL distilled water was placed in a 500 mL round bottom flask. Ten grams of fish were added in the flask, then 2.5 mL of 4M HCl and 1.5 mL BHT were added to prevent autoxidation. In addition, 10 drops of silicon oil and 5 glass boileezers were added to the flask. The mixture was distilled until collection of the first 50 mL distillate and 5 mL of this distillate were placed into a glass vial containing 5 mL of 0.021M aqueous solution of thiobarbituric acid and 0.6mL of BHT solution. The mixture was placed in a water bath for 40 minutes at 90 ^{0}C until a pink complex was developed. The solution was left to cool then it was filtered using Teknokroma^{TM} PVDF (=polyvinylidene difluoride) filters 0.45 μm.

A blank was prepared by adding 5 mL water in 5 mL TBA and 0.6 mL BHT solution. The solution was filtered and the absorbance was measured at 532 nm. The value of MDA was then calculated as follows (Tarladgis et al., 1964):

mg MDA/kg = absorbance x 7.8 …………………………………….. Equation (5)

3.6.   Determination of surface energy using water contact angle device

The contact angle of a water droplet on the differently treated film surfaces was measured using a Kruss drop shape analyzer DSA25B with the mechanism of static sessile drop method. Measurements were carried out at 20 ^{0}C and five readings were taken for each type of film. The average was calculated for each set of readings.

3.7. Determination of surface morphology using scanning electron microscopy (SEM)

Films (1x1 cm) were cut and gold sputtering with Hummer 8 gold sputter was performed on the film surfaces to enhance conductivity. SEM images were taken by Leo Supra 55 microscope. Images were taken at a magnification of 1700 X and resolution of 10 μm.

3.8. Determination of chemical characteristics using Fourier Transform Infra Red (FTIR) spectroscopy

Small pieces of the differently treated films (1x1 cm) were cut and inserted in the beam path of Thermo scientific FTIR model: Nicolet 380 to identify functional groups (bonds) on the film surface in the range of 400 to 4000 cm^{-1}.

3.9. Determination of surface groups using X-ray photoelectron spectroscopy (XPS)

XPS experiments were performed on a model Axis Ultra DLD system by KRATOS. A monochromated Al-Ka X-ray beam was used as the excitation source. A pass energy of 20 eV was used resulting in a full width at half maximum of the Ag-3d5/2 line less than 500 meV. Data interpretation was performed with the Kratos-Vision software (Version 2.2.10).

3.10. Determination of oxygen permeability

The test was performed twice on chemically modified PET films without coating, using an oxygen permeability tester model: Oxtran 2/20 Mocon Co, Minnesota, USA. The instrument was set to convergence mode where two successive readings were taken with no more than 5% difference. The temperature of instrument during test was 23°C.

Chapter 4

Theoretical background

4. Theoretical background

4.1. Scanning electron microscopy (SEM)

Scanning electron microscopy is an instrumental analytical method of determining surface morphology and sample crystallinity. This is done through producing a high energy beam of electrons that interact with the sample surface. The energy of electrons is then dissipated - after electrons are decelerated by the sample surface - in the form of secondary electrons, backscattered electrons, diffracted backscattered electrons, photons and heat. Secondary electrons and backscattered electrons are responsible for image formation of surface topography and illustrate contrasts in multiphase samples, respectively. Backscattered electrons are produced when electrons from the source collide with the sample elastically losing very small energy and reflecting back with energy close to that of the initial beam while secondary electrons are produced when source electrons collide with the sample in-elastically causing ionization of the sample and the emitted or secondary electrons are of much less energy than that of the source electrons (McMullan, 2006)

Components of the instrumentation include (Khursheed, 2011): electron gun, electron lenses, data output device, sample stage, detectors, vacuum system, cooling system, power supply, vibration free floor and a room free of ambient electric and magnetic field. Electrons in the electron gun are produced by heating a tungsten filament that has voltage applied to it serving as the cathode. The electrons produced are accelerated toward the anode and move back and forth - by a set of electron lenses - striking the sample surface. The reflected electrons are used to form an image on a computer monitor (Fig. 14).
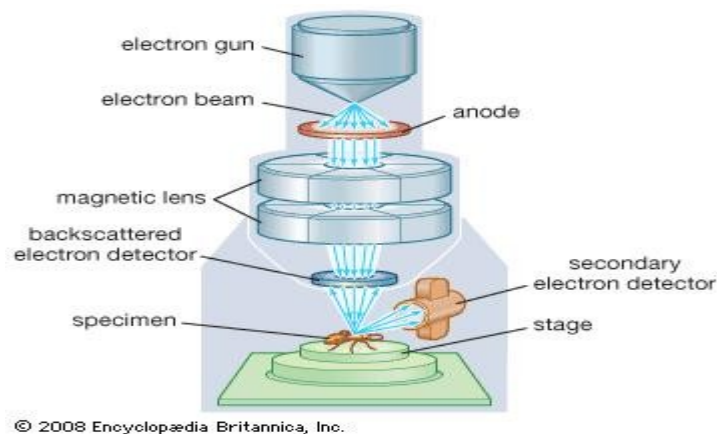
Fig. (14). Components of scanning electron microscope (Ref: Encyclopaedia Britannica - SEM.)

The instrument is used widely in the study of solid materials as it has much higher resolution than optical microscopes since electrons have much shorter wavelengths than light, however it has several limitations (Zhou & Wang, 2007). These limitations include the small sample size being of about 10 x 4 cm. Another limitation is that samples that produce gases such as rocks producing hydrocarbons or wet samples as coal cannot be determined by SEM unless certain types of SEM are used. Also, certain samples are electron insulating which cannot be used unless they are coated - by specific instruments – by carbon or gold to prevent the building of charges over the sample. Carbon coating is used when elemental analysis is required while gold coating is used to produce high resolution images.

3.2. FTIR (Fourier Transform Infrared Spectroscopy)

Infrared spectrometers are used to identify the functional groups present in a certain material. Unlike UV/VIS spectroscopy that depends on excitation of electronic energy levels, they depend on the excitation of vibrational energy levels. These levels are closer to each other than are the electronic energy levels. Thus, the energy used to excite a sample is much less than that used for UV/VIS spectroscopy (IR ranges from 800 nm to 1 mm or $400 – 4000$ cm^{-1}).

56

Absorption of energy is quantized and depends on the fact that the molecule has an electrical dipole moment changing at the same frequency of the transferred energy. Thus, this technique is used for organic or inorganic compounds having covalent bonds. The vibrational motion of bonds can be classified into symmetric, asymmetric and bending. Asymmetric vibrations have higher frequencies than symmetric ones which, in turn, have higher frequencies than bending vibrations. (Smith, 2011)

FTIR is a more sophisticated method compared to the older dispersive IR. In the older method (Fig. 15), light produced from a hot wire is split so that one beam passes through the reference or blank cell and the other passes through the sample. The two transmitted beams then combine together and the transmitted light passes through a monochromator that disperses light into its colors or frequencies. These frequencies are then focused on a detector giving a profile for the amount of energies that was absorbed by the sample. The spectrum is in the form of intensity vs. frequency or wavenumber. (Smith, 2011)
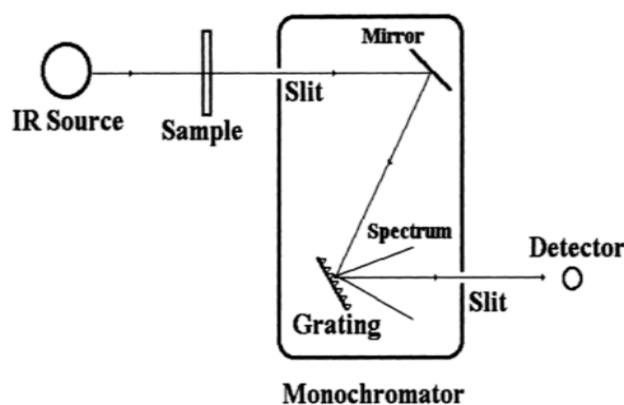
Fig. (15). Diagram showing dispersive IR spectrometer operation (Smith, 2011).

In case of FTIR (Fig. 16), the beam (usually from a broadband laser) is split by a beam splitter into two beams, one passes to a fixed mirror and the other passes to a mobile mirror – perpendicular to the fixed one - in a section called interferometer. These two beams then

57

combine and pass through the sample then to the detector. The combination of the two beams may result in a destructive or constructive interference depending on the distances moved by the mobile mirror. The resulting data are decoded by a computer software (performs Fourier transformation) as they are a collection of all IR frequencies together as a function of the mobile mirror. Thus, FTIR is better than the older method in that it is faster (measures the complete spectrum of frequencies at one time), simpler because there is only one mobile part therefore less tendency for mechanical breakdown, self-calibrating and more sensitive. (Smith, 2011)
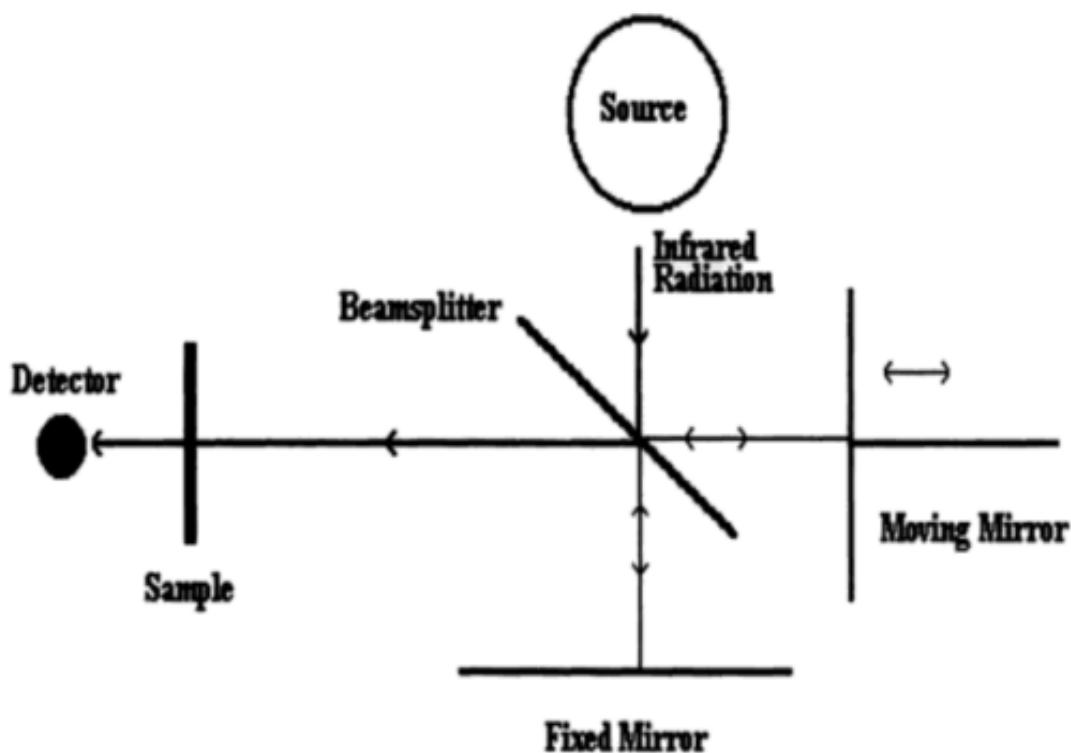
Fig. (16). Diagram showing FTIR operating principle (Smith, 2011)

It is to be noted that the cuvettes made for FTIR are made from ionic compounds and this guarantees no interference with the sample energy absorption. These compounds might be

sodium chloride or potassium bromide. Sodium chloride is cheaper but cannot be used for all absorption bands because it absorbs energy at 650 cm^{-1}. However, potassium bromide is inert at all frequencies.

Liquid samples are prepared by inserting a drop of liquid in between two plates of sodium chloride or potassium bromide forming salt plates where no solvents are added. In case of solids, there are several methods to prepare the sample. The first one involves mixing the solid sample powder with potassium bromide and subjecting the mixture to pressure forming potassium bromide pellets. The second one involves mixing the solid sample with mineral oil and placing the formed suspension between salt plates. However, the third one involves dissolving the sample in carbon tetrachloride and measuring it (Zofka, 2013)

3.3. UV/VIS Spectroscopy

UV/VIS spectroscopy that is used in both DPPH and TBA is a type of spectroscopic measurement that depends on the excitation of electrons in the electronic levels to a higher state by light of wavelength range from 200 to 800 nm. The amount of energy absorbed is quantized and proportional to the concentration of the sample according to Beer-Lambert law. Electronic transitions usually occurs from σ to σ*, n to σ*, n to π*, π to π* where σ electrons are those of a single bond and pi electrons are of double bonds while n electrons are of the lone pairs. Sigma transitions usually need high amounts of energy which will be only supported by ultraviolet waves. Transitions usually occur from the highest occupied molecular orbital to the lowest unoccupied molecular orbital. Electronic transitions depend on the electronic structure of the sample. For example, there are chromophores that can induce shifts in wavelength absorption especially when conjugated. Shifting absorption to a higher wavelength is called bathochromic shift while shifting absorption to shorter one is

hypsochromic shift. Other factors also affecting absorption are the type of solvent used for the sample and pH (Perkampus, 1992).

The working principle of the spectrometer (Fig. 17) involves that light at 200 to 800 nm is produced from a deuterium lamp that passes through a monochromator to disperse light then enters a beam splitter. The beam after going through the beam splitter is split into two beams one passing through the sample cuvette (usually made of quartz) and the other through the blank. The transmitted light then hits the detector that converts it into a current. The higher the current produced the greater the intensity. UV/VIS spectroscopy is used in several applications. For example, it is used in clinical chemistry to study enzyme kinetics. It is also used in pharmaceutical industry to quantify the amount of drug released after dissolution testing. It can be used in genetics to quantify DNA or protein/enzyme activity and it can be used in the quality control of dyes and paints (Clark et al. 1993)
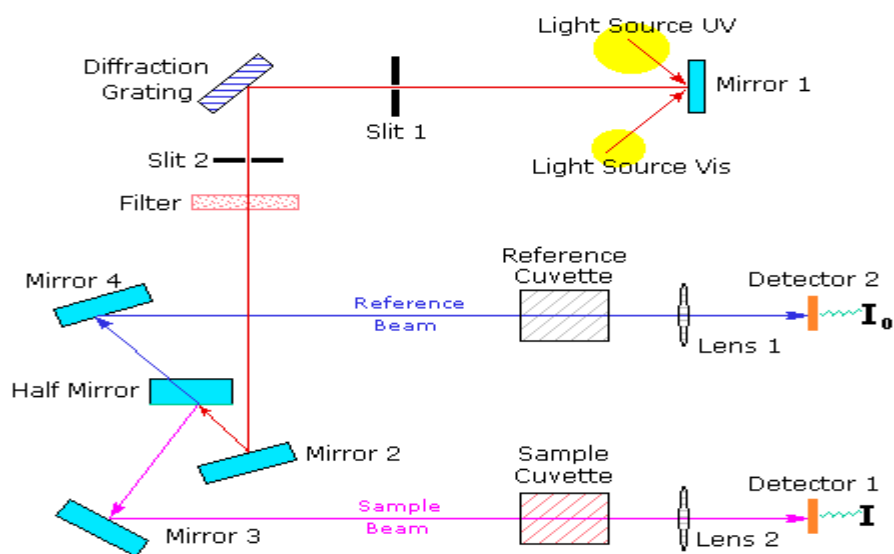
Fig. (17). Diagram showing UV/VIS spectrometer (Ref.: MSU university: UV/VIS spectroscopy)

3.4. X-ray photoelectron spectroscopy (XPS)

It is a form of spectroscopy that is used in elemental analysis of the solid surface at depth 1 –
10 nm. It is also used in determining the chemical and electronic states of the material. The
theory behind this instrument is that an x-ray photon is directed to the surface of the material
and an electron is ejected. This occurs via energy transfer from the photon to the electron
where the energy of the emitted electron is equal to its binding energy which is characteristic
to the element analyzed (Van der Heide, 2011).

XPS is mainly composed of X-ray source, extraction optics or analyzer with energy filter and
a detection system (Fig. 18). The instrument works under vacuum which is important for
minimizing the probabilities of electron collision with any suspended atoms and thus enhances
transmission and detection. The extraction analyzer is usually a spherical deflection analyzer.
This consists of two concentric hemispheres and its resolution power is proportional to the
radius of the inner and outer hemispheres (Hufner, 2013).
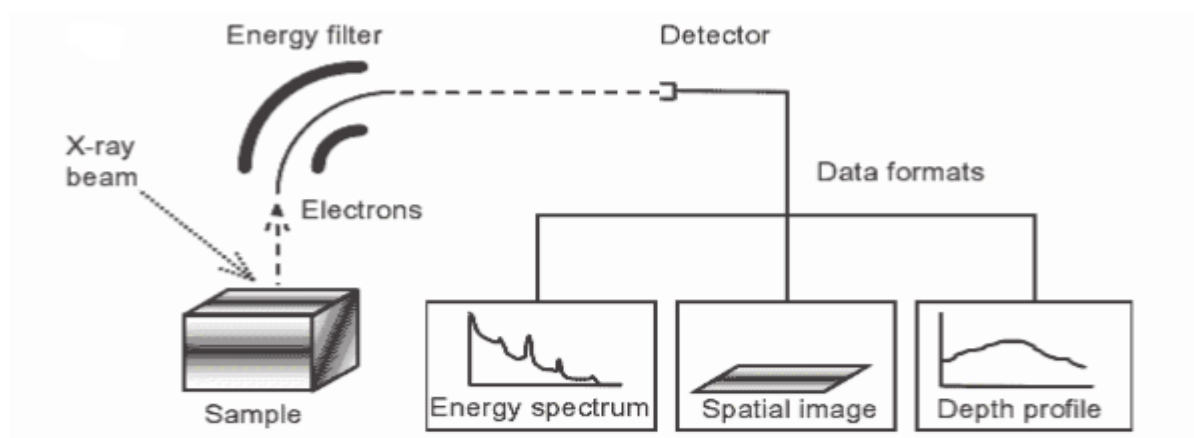
Fig. (18). Diagram showing XPS instrument (Van der Heide, 2011)

The detector in XPS is an electron multiplier. Its role is to magnify the produced signal. This occurs via a material by which it is coated and this material has the ability to produce extra electrons when they are struck by the incoming electrons (Hufner, 2013).

XPS has several applications through its surface analysis capability. This is important in studying corrosion, adhesion, fatigue, coatings, barrier layers, catalysis, ..etc. However, it has limitation in analyzing hydrogen and helium atoms because their photo-electron cross section is very low to be detected by XPS (Yakacki & Gall, 2009)

3.5. Oxygen permeability tester

The oxygen permeability test is performed according to ASTM method D3985. The method is called isostatic method in which the instrument is designed so that there are two chambers separated by the tested film (Fig. 19). One chamber has a flow of oxygen rich gas which permeate the film to the second chamber. The second chamber has a flow of a carrier gas that directs the permeated oxygen to the oxygen sensor. In this method, the pressure and flow rate of the gas are equal in both chambers and hence the name isostatic. The oxygen sensor used in this instrument is a coulometric sensor that expresses the amount of reaching oxygen as an electric current proportional to the amount of oxygen per unit time. It is very sensitive to detect the smallest amount of oxygen in an oxygen-free carrier gas with the lowest noise (Ref: Diamon^{TM} Labthink company: Standard for coulometric film oxygen permeability testing).
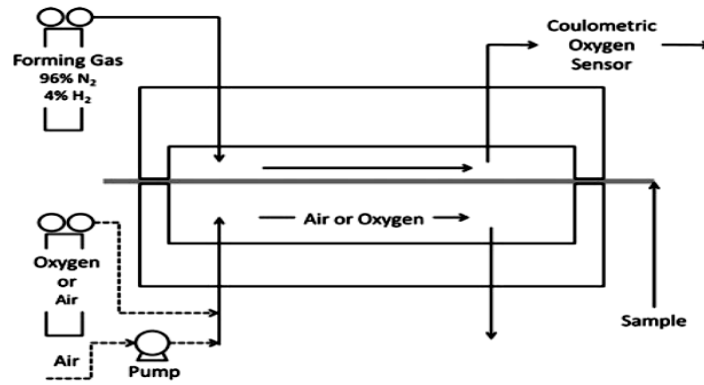
Fig. (19). Diagram showing an oxygen permeability tester (Abdellatief & Welt, 2013)

Chapter 5
Results and discussion

5. Results and discussion

5.1. DPPH scavenging activity

Before determining the antioxidant activities of the coated films, DPPH scavenging activities of the rosemary and clove extracts were first determined.

Figs. 20 and 21 show the change in % RSA and % remaining DPPH with concentration of the rosemary and clove extracts, respectively.
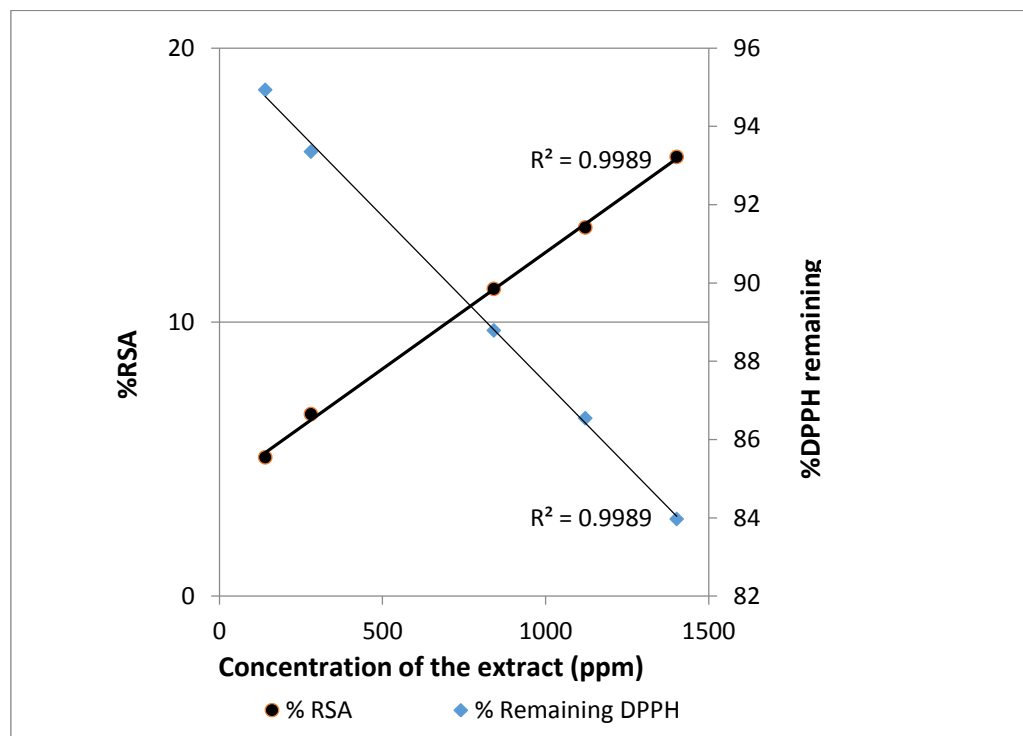
Fig. (20). %RSA and %DPPH remaining as a function of concentration of rosemary extract.
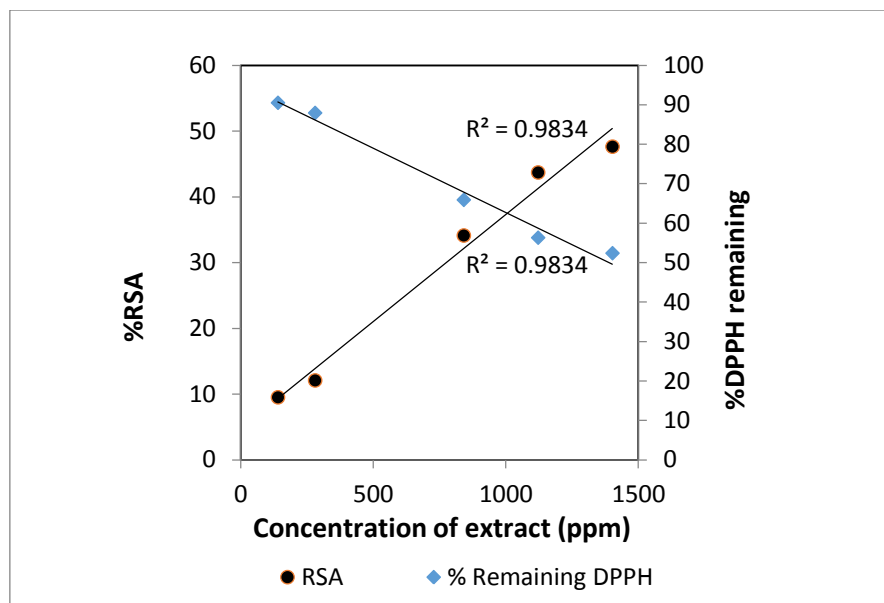
Fig. (21). %RSA and %DPPH remaining as a function of concentration of clove extract.

The figures show that by increasing the concentration of the extract, the percentage remaining DPPH decreases while the % RSA increases. In addition, it is clear that rosemary and clove extracts show potent radical scavenging activities with clove having higher activities than rosemary.

Values for antioxidant activity of rosemary extract are comparable to those in the literature. Viuda-Martos et al. (2010) determined the radical scavenging activity of rosemary extract at a concentration of 20000 ppm for rosemary extract and reported to be 11.28%. This value is close to that obtained in this study at 841.2 ppm. These results show that rosemary used in our study is much more potent than that used by Viuda-Martos in his study. Differences in antioxidant activity may be due to differences in rosemary cultivars harvesting practices used, as well as variation in soil conditions across various geographical regions. Ali Hassan et al. (2012) compared the antioxidant activity of Egyptian rosemary to that of the Malaysian source. He found that at 1000 mg/L, the radical scavenging activity for the Egyptian rosemary

66

was 24.7%. This value is higher than that obtained in the present study, where the radical scavenging activity was 16.02% at a concentration of 1402 mg/L.

In another study, Giorge et al. (2014) investigated the antioxidant activity of the acetone and hydroalcoholic extracts of rosemary. It was found that the acetone extract had a radical scavenging activity of 4.98% while the hydroalcoholic extract had an RSA of 20.1% at 1000 ppm. This indicates that the ethanolic extract had a lower radical scavenging activity than the hydroalcoholic extract with a decrease of 20.29% but had a higher scavenging activity than acetone extracts with an increase of 68.91%.

In case of clove, Dudonne et al. (2009) reported the radical scavenging activity of clove extract to be 31.58%, however they did not mention the concentration of extract at which this value was measured. This value is close to that measured in our work (34.11% at 841.2 ppm), with our value being higher by 7.4%.

Following is the study for the determination of the antioxidant activities of the extracts pertaining to the plasma treated, corona treated and chemically modified PET films.

The %RSA and %DPPH remaining as a function of concentration of the rosemary coated films and rosemary extract are depicted in Figs. 22 and 23, respectively.

Fig. (22). %RSA as a function of concentration of the treated films coated with rosemary, along with values pertaining to rosemary herbal extract

Fig. (23). %DPPH remaining as a function of concentration of the treated films coated with rosemary along with values pertaining to rosemary herbal extract

The figures show that %RSA increases gradually while %DPPH remaining decreases gradually by increasing the concentration. As clear from Fig. 21, the extracts of the plasma treated films with rosemary coating have the highest % RSA followed by surface corona

68

treated films then chemically modified films. This may probably be attributed to the lower adhesion of rosemary onto the plasma treated films compared to the other two treatments resulting in a higher release of antioxidant compounds from the film to the extracting solution. As for the %DPPH remaining (Fig. 22), the lowest value corresponds to the plasma treated films.

This general trend was not only shown in % RSA but also in the antiradical efficiency (AE). AE for plasma treated, corona treated and chemically modified films were 0.35, 0.28 and 0.26, respectively.

As for clove treated films, %RSA and %DPPH remaining are shown in Figs. 24 and 25, respectively. Fig. 23 shows that %RSA for all treated films are comparable at the different employed concentrations. Furthermore, % RSA values for the film extracts are lower than those of the herbal extract. Consequently, the %DPPH remaining values of the coated films are higher than those of the extract (Fig. 24). This may possibly be due to the excessive evaporation of clove during film preparation which along with the results of contact angle measurements, that will be later discussed, explains this trend. The antiradical efficiencies for the clove coated plasma treated films (3.08) were found to be somewhat lower than those of the corona treated (3.31) and chemically modified films (3.24).

For both rosemary and clove coated films, it was found that the %DPPH remaining decreases gradually while %RSA increases gradually by increasing the concentration (Figs. 21 - 24). By comparing the radical scavenging activity results of rosemary and clove, it can be observed that all films gave comparable %RSA values ranging from 5 to 25%, although clove coated films could have exhibited higher activity but the excessive evaporation that occurred during film preparation resulted in decreased activity.
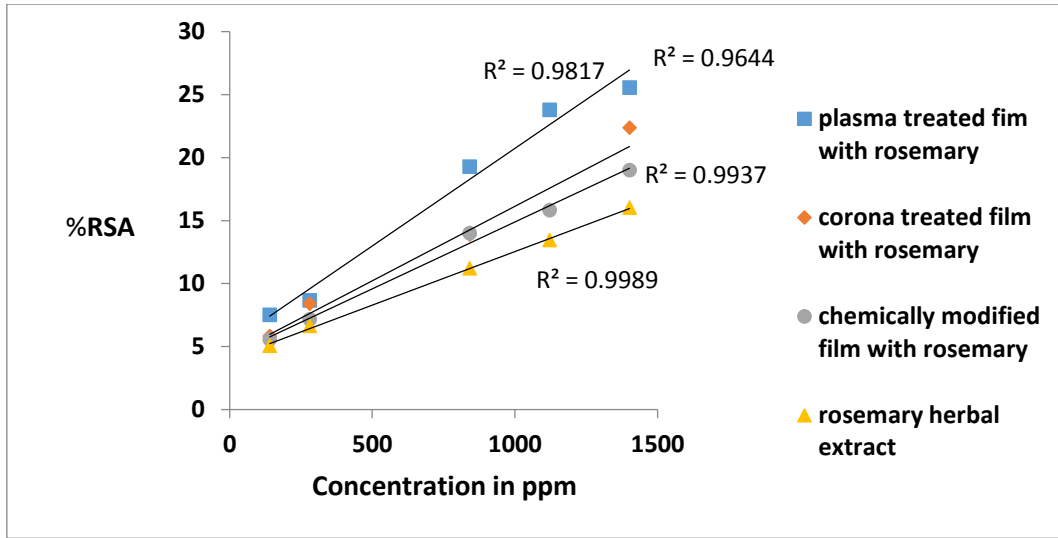
69
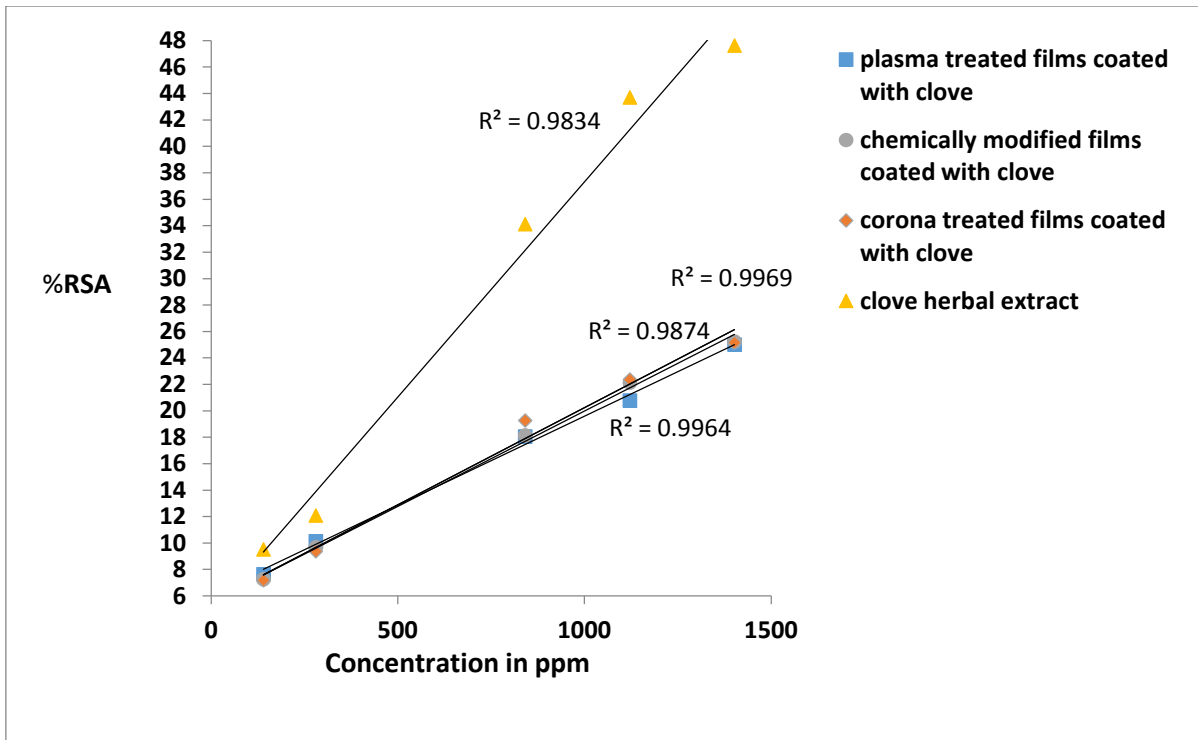
Fig. (24). %RSA as a function of concentration of the treated films coated with clove, along with values pertaining to clove herbal extract

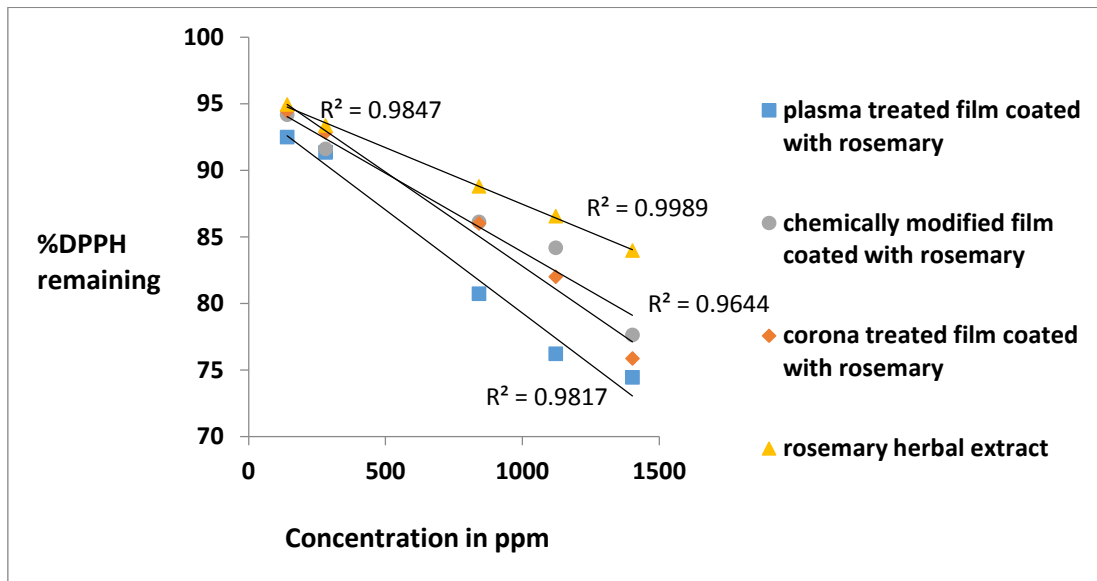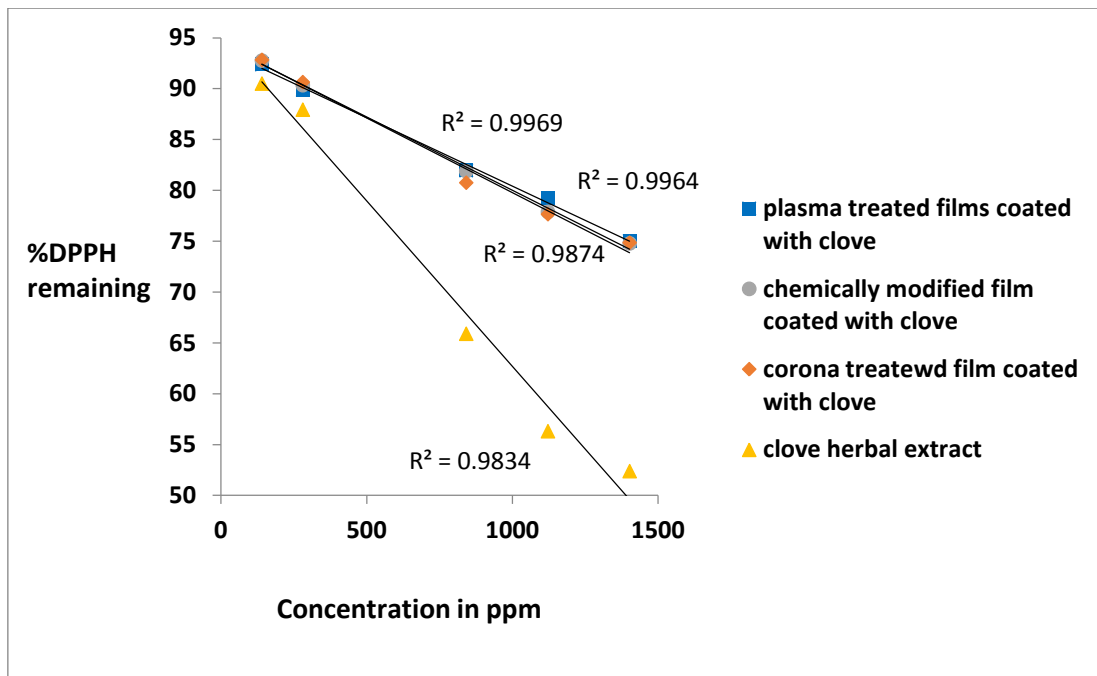Fig. (25). %DPPH remaining as a function of the concentration of the treated films coated with clove along with values pertaining to clove herbal extract

70

Values of IC$_{50}$ for rosemary and clove coated films are presented in Table 4. These values confirm that chemically modified films have the most potent antioxidant activity among other rosemary coated films. All the clove coated films, on the other hand, showed comparable antioxidant activities irrespective of their treatment.

Table (4): IC50 values (expressed as mg/ml) for the rosemary and clove coated films with different treatments.

	Rosemary coated chemically modified films	Rosemary coated corona treated films	Rosemary coated plasma treated films	Clove coated chemically modified films	Clove coated corona treated films	Clove coated plasma treated films
IC$_{50}$	3.87	3.33	2.89	3.09	3.02	3.25

An interesting observation was that % RSA values for the coated films were higher than those of the extracts (Table 5). This is most probably due to the extraction of antioxidants that were compounded into the PET films during manufacture.

Table (5): %RSA of PET film extract (without coating)

Concentration in ppm	%RSA
140.2	5.72
280.4	5.96
841.2	5.78
1121.6	5.39
1402	5.72

5.2. Total viable count (TVC) measurements

This analysis is carried out to determine the time at which the TBA test should be discontinued (time to reach a total viable count of 7 log cfu/g of fish muscle) as it would be meaningless to test the antioxidant activity once the substrate (fish) is considered microbiologically spoiled (De W. Blackburn, 2006).

Our results showed that the experiment should be terminated on day 6 (Table 6) where the count was on the borderline of microbiological spoilage that is equal to 7 log cfu/g. Based on this finding, we chose to conduct the TBA test during the first six days of storage.

Table (6): TVC values of packaged ground fish on day 6 of storage

Type of film	Log cfu/g
Untreated uncoated control PET film	6.65 ±2.6
rosemary coated corona treated film	5.5 ±0.52
rosemary coated chemically modified film	5.34 ±0.36
clove coated corona treated film	6.57 ±1.36
clove coated chemically modified film	6.57 ±1.3
Rosemary coated plasma treated film	5.17 ±0.20
Clove coated plasma treated film	5.29 ±0.28

5.3. TBA antioxidant activity

Table 7 and Figure 26 show the TBA results pertaining to rosemary coated PET films in contact with ground fish, along with the control film TBA values.

Table (7): Absorbance values of mackerel distillates for corona treated, plasma treated, chemically modified films coated with rosemary along with their control

Day	Untreated uncoated control PET film	corona treated film coated with rosemary	Chemically modified film with rosemary	Plasma treated films with rosemary
0	0.3796 ±0.132	0.2325 ±0.026	0.488 ±0.032	0.4175±0.038
4	1.798 ±0.094	0.3775 ±0.0715	0.731 ±0.111	NA[*]
6	2.052 ±0.171	0.558 ±0.006	0.605 ±0.039	0.345±0.009

* NA = not analyzed
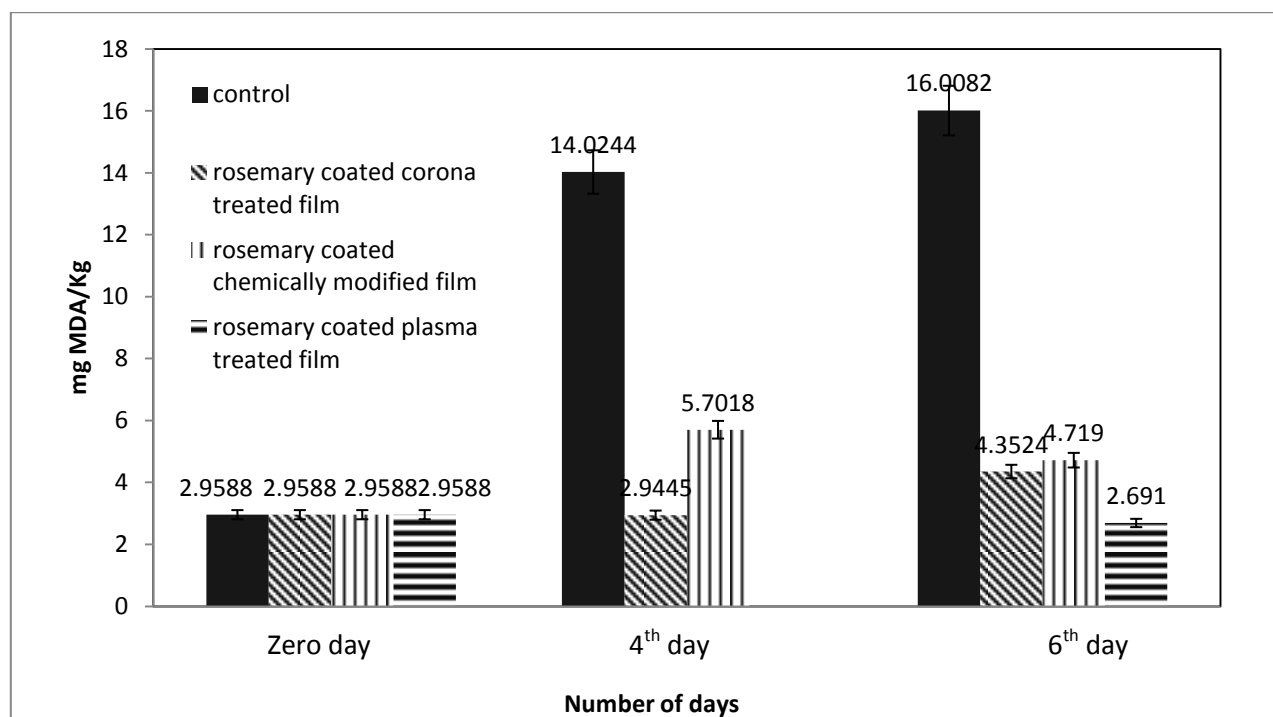
Fig. (26). Values for mg MDA/Kg for packaged fish in rosemary coated corona treated, chemically modified, plasma treated films along with control

Compared to the control film, all films showed a considerable protection of fish against

oxidation. The corona treated PET films coated with rosemary showed 79.0% reduction in the

degree of oxidation on day 4 and 72.8% reduction in the degree of oxidation on day 6 compared to the control film on the same day. Regarding the chemically modified films coated with rosemary, the percentage reduction of oxidation of the fish muscle compared to the control of the same day was 59.3% on day 4 and 70.5% on day 6. It should be noted that mg MDA/kg of fish decreased on day 6 compared to day 4. This may be due to the fact that malondialdehyde is not a stable molecule which degrades upon further reaction with aminoacids and proteins (Sochr et al., 2014;Kostka et al.,1989).

Ozogul et al. (2010) measured the TBA values for vacuum packaged sardines treated with 1% and 2% rosemary of fish weight. It was found that on day 6, 1% rosemary was able to reduce the degree of oxidation by 63.68% while 2% rosemary decreased the degree of oxidation by 67.59%. These findings are comparable to results obtained in this study, regarding the efficiency of rosemary to reduce oxidation compared to the control film, where in this study rosemary coated films (having rosemary of 0.28% of the fish weight) caused reduction in oxidation by 72.8% and 70.5% for corona treated and chemically modified films, respectively. Kenar et al. (2010) reported TBA values for sardines after addition of 10g/L rosemary extract and found that degree of oxidation was reduced by 70.0% on day 3 and 61.0% on day 6 relative to the control. These values are close to values obtained in this study.

Barbosa-Perriera et al. (2014) studied the TBA values of beef spiked with 0.05% and 3% rosemary extract. They also studied the effect of LDPE package activated with rosemary of concentrations 3%, 10% and 20% on the beef patties. For 0.05% rosemary, degree of oxidation was reduced by 32% and 36% on day 3 and 7, respectively. As for the 3% concentration of rosemary, the reduction in degree of oxidation was 49.16% and 49.49% on day 3 and 7, respectively. Regarding the films, 3% rosemary containing films showed 44.57%

and 58.29% reduction in the degree of oxidation on day 3 and 6, respectively. Whereas 10% rosemary film exhibited 73.11% and 70.59% reduction in the degree of oxidation on days 3 and 6, respectively. The 20% rosemary film showed 70.0% and 81.19% reduction in degree of oxidation on day 3 and 6, respectively. Comparing the 10% rosemary film to that in the present study, prepared to contain also10% rosemary, it is clear that both are very comparable to each other regarding antioxidant activity, especially on day 6 of refrigerated storage.

Table 8 and Figure 27 show the TBA results pertaining to clove coated PET films in contact with ground fish, along with the control film TBA values.

Table (8): Absorbance values of mackerel distillates for corona treated, chemically modified, plasma treated films coated with clove along with their control

| Day	Untreated control PET film	Clove coated corona treated film	Clove coated chemically modified film	Clove coated plasma treated film
0	0.3795±0.053	0.3415±0.0005	0.3415±0.0005	0.4175±0.038
4	1.415±0.137	0.5165±0.024	0.482±0.007	NA[*]
6	2.3212±0.5504	1.0615±0.296	1.0845±0.488	1.662±0.335

* NA = not analyzed
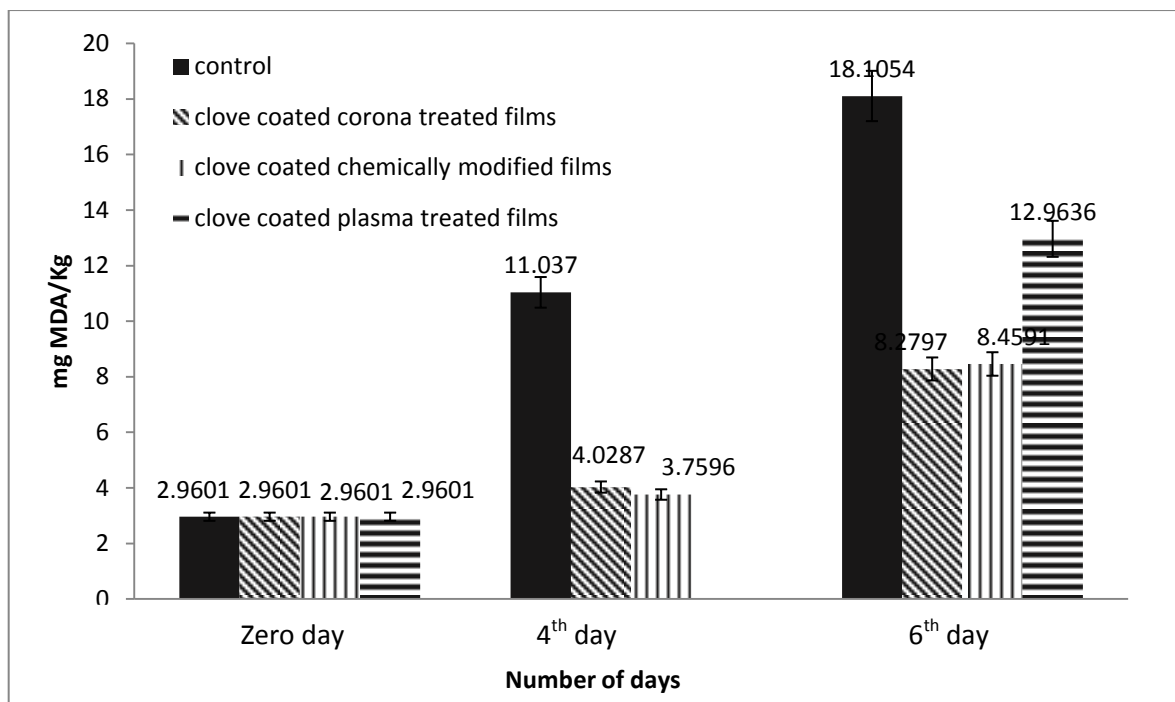
Fig. (27). Values for mg MDA/kg for packaged fish in clove coated corona treated, chemically modified, plasma treated films along with control

The corona treated films showed 63.5% reduction on day 4 and 54.3% on day 6 in the degree of oxidation compared to the control films of the same day, while the chemically modified films showed 65.9% reduction in oxidation on day 4 and 53.3% on day 6. In case of plasma treated films, clove showed a reduction of only 28.4% in oxidation of fish muscle compared to the control film on day 6.

Guran et al. (2015) determined the TBA values for bonito fish having clove and rosemary essential oils added to them. They found that the fish with no antioxidants showed 523.2% oxidation increase from the initial (day zero) on day 4. Respective increase in degree of oxidation from the initial was 302.56% and 215.15% on day 4 for both clove and rosemary. In our work, there was no increase in the degree of oxidation on day 4 for the corona treated films coated with rosemary but the increase in the degree of oxidation was 71.41% for coated

chemically modified films compared to the control that gave 373.98% increase in the degree of oxidation (Fig. 26). For clove, the increase in degree of oxidation on day 4 was 36.1% and 27.01% for the clove coated corona treated films and clove coated chemically modified films, respectively compared to 272.85% for the control film. (Fig. 27)

Yang et al. (2016) performed the TBA test on grass carp slices packaged in ethylene vinyl alcohol films activated with clove. Results showed that on day 4, 32.75% reduction of oxidation occurred to the clove activated films compared to the control film followed by 30.13% reduction in degree of oxidation on day 6. These values are much lower than those obtained in the present work which is understandable in view of the difference in the type of fish tested, quality of the clove used, type of film, ..etc.

It can be concluded that for all types of films, percentage reduction in the degree of oxidation varied from 59.3% up to 79.0% on day 4 relative to the control of the same day, while on day 6 it ranged from 53.3% to 72.8% except for clove coated plasma treated films that showed a 28.4% reduction in oxidation.

Thus, results show that both rosemary and clove coated films have the ability to extend the shelf life of fish with regard to oxidation. Of the two antioxidant agents rosemary and clove, the first proved to provide a higher antioxidant activity. This fact may be related to the specific composition of the two agents.

77

5.4. Water contact angle measurements

Water contact angles for the corona treated, plasma treated and the chemically modified films, along with the untreated control film are shown in Table 9.

Table (9). Contact angle measurements for corona, chemically modified and plasma treated films along with the control

Film type	Contact angle ^{0}
Untreated film	76.93 ± 5.57
Corona treated film	53.69 ± 6.32
Plasma treated	63.78 ± 3.41
Chemically modified	31.13 ± 9.42

*Values are given as mean value±SD (n=5)

It is shown from the above results that chemically modified films are the most hydrophilic and have the highest surface energy and thus adhesion capacity. The order of film surface energy followed the sequence: chemically modified film>corona treated film>plasma treated film> untreated film.

Yangchuan et al. (2010) reported the contact angle for untreated PET to be 72.0^0. This value is close to that measured in the present study.

5.5. Surface morphology by scanning electron microscopy

Figures 28 (a - d) depict SEM images pertaining to the untreated (a), corona treated (b), chemically modified (c) and plasma treated (d) PET films.
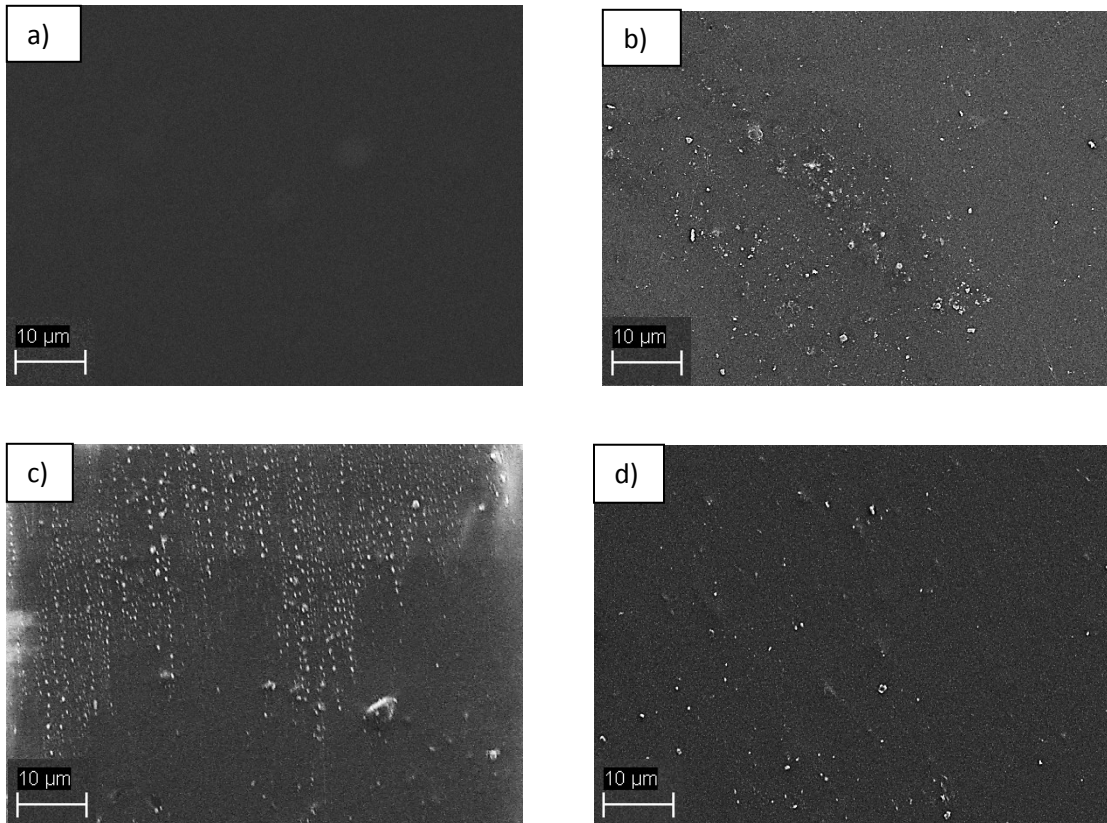
Fig. (28). SEM micrographs of (a) untreated, (b) corona treated, (c) chemically modified, (d) plasma treated films.

The figures show that the untreated film has a very smooth surface assuring no surface treatment for that film. However, the three other treated films show surface roughness of variable degrees. The degree of roughness is indicated by the number of surface anomalies formed. These anomalies provide evidence of the physical change of the film surface. The chemically modified film shows the highest surface roughness followed by corona treated film

then plasma treated films. These results are in alignment with contact angle results for surface roughness.

Ding et al. (2014) studied the effect of corona treatment on the surface of PET films. He found with the aid of X-ray photoelectron spectroscopy, atomic force microscopy and SEM that new surface groups are formed and that the surface roughness increased after corona treatment as was indicated by SEM microscopy which produced images similar to those of the present work.

Sun et al. (2010) plasma treated PET (helium/oxygen gas) for graft polymerization reactions with acrylic acid. They treated the films for 30, 90, and 180 seconds. It was shown that by increasing the time of plasma treatment, lamellar structures appear. These structures provided evidence of film surface etching. After plasma treatment of the films, graft polymerization with acrylic acid was performed by the inverse emulsion technique. SEM images showed the appearance of large patches. The reason behind this could be attributed to the generation of free radicals by plasma treatment which begin chain reaction with acrylic acid monomers. The formed chains propagate and increase in size. As long as the distance between the initial free radicals is small and that the size of chains increases, they begin to overlap forming patches that appear in the SEM images.

5.6. FTIR analysis

Figures (29-32) depict FTIR images pertaining to the untreated, corona treated, chemically modified and plasma treated PET films, respectively.
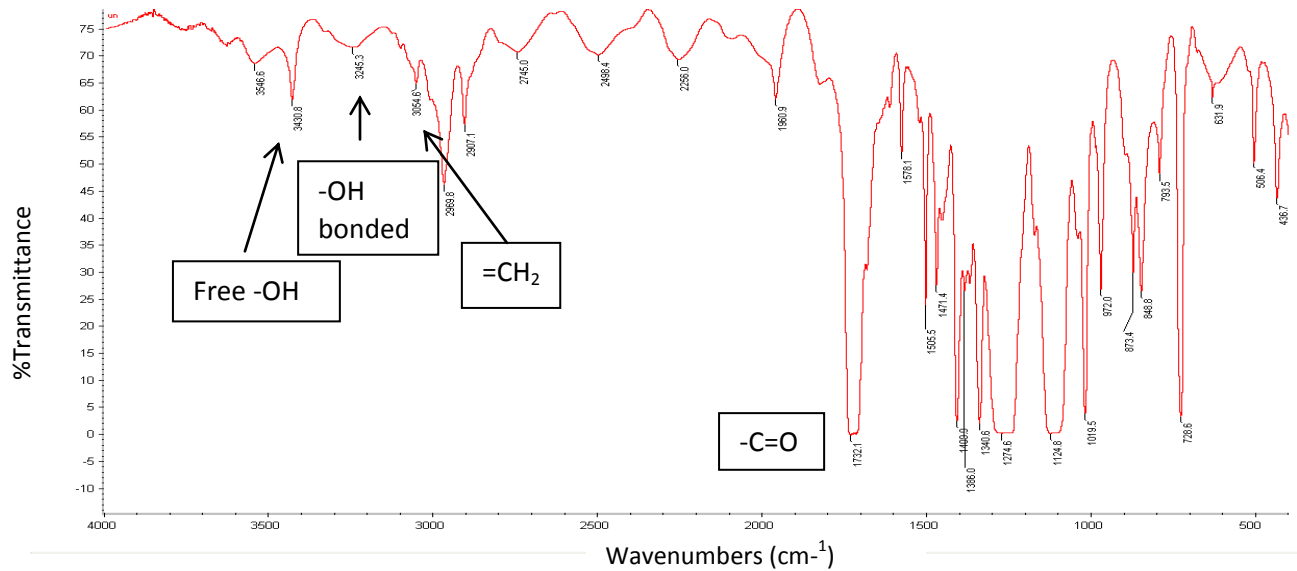
Figure (29): FTIR spectrum of untreated PET film.
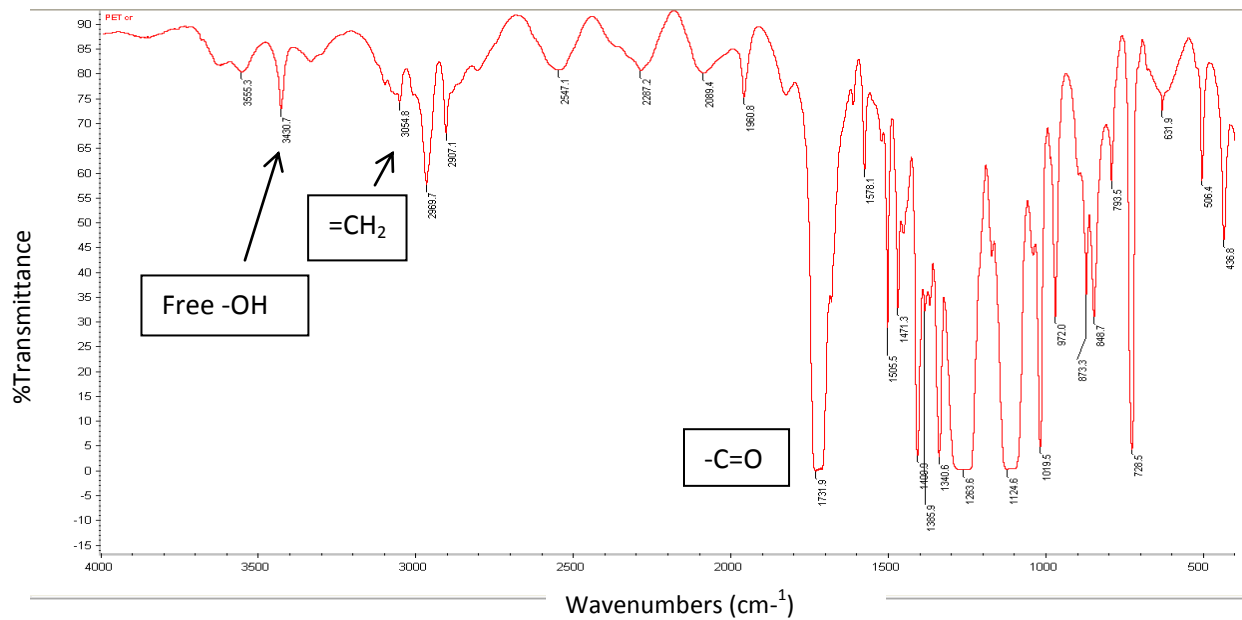
Figure (30): FTIR spectrum of corona treated PET film.
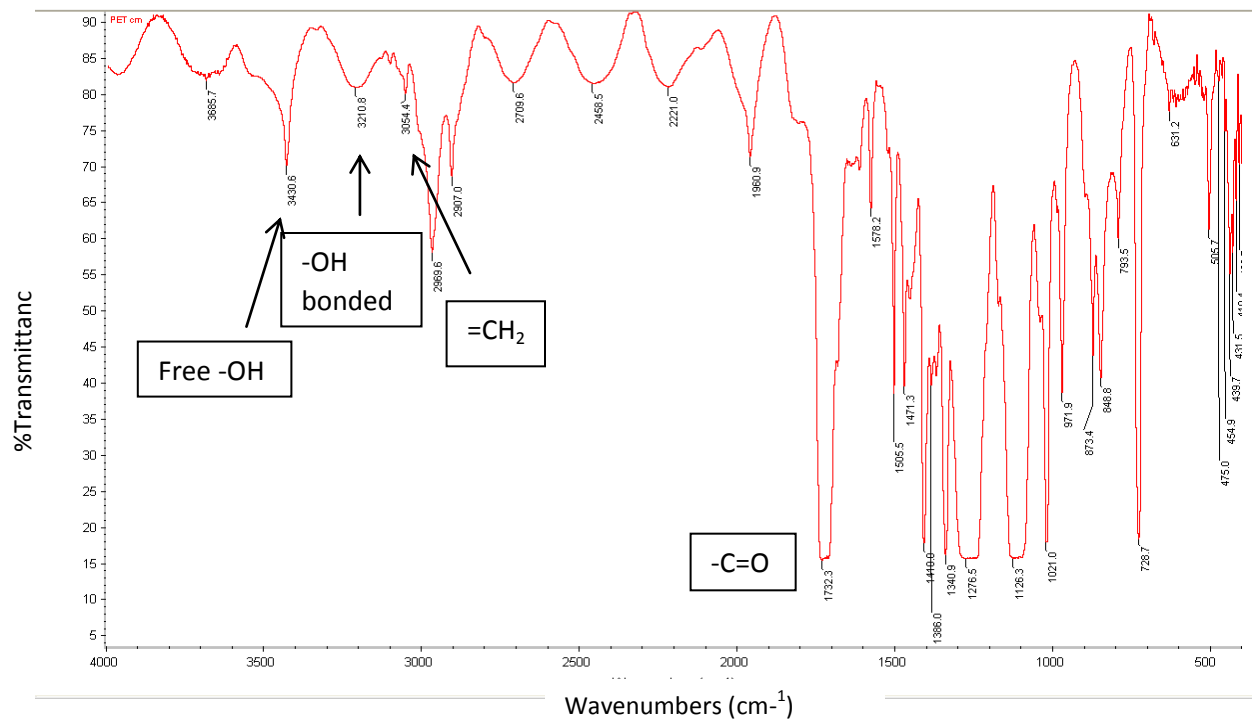
Figure (31): FTIR spectrum of chemically modified PET film.
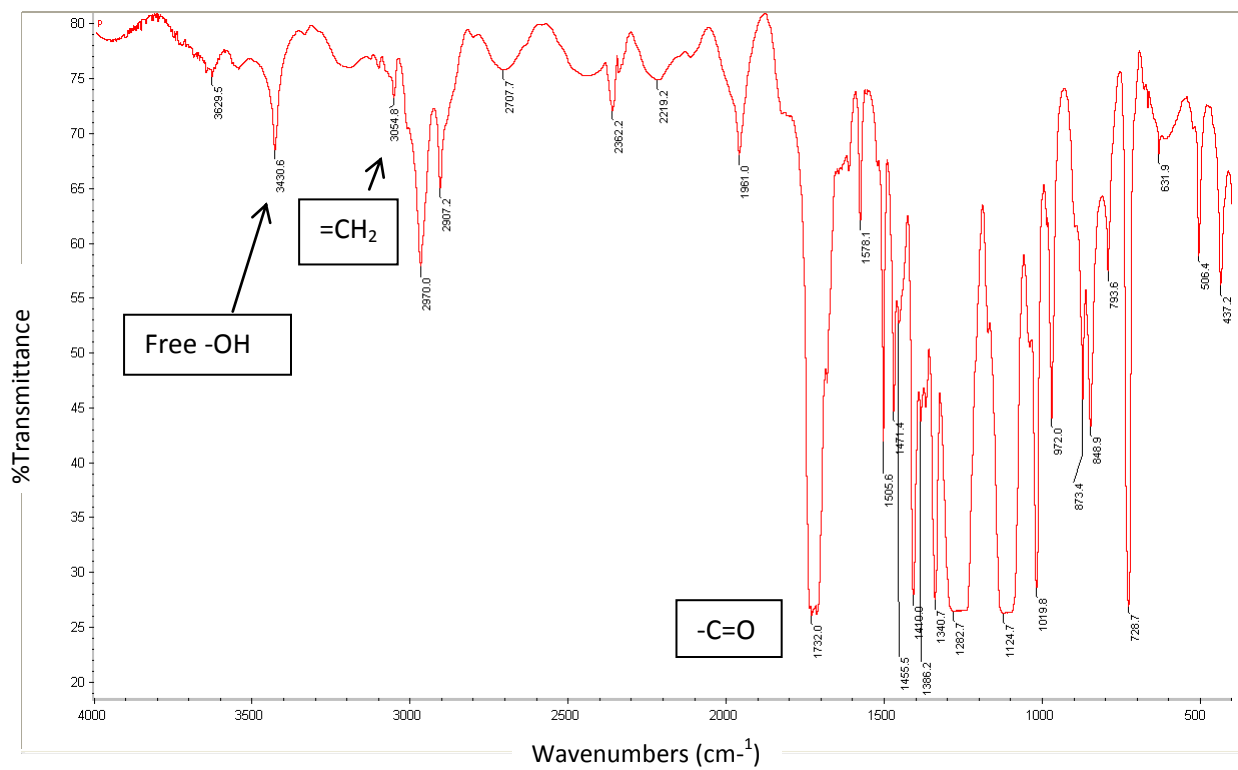
Figure (32): FTIR spectrum of plasma treated PET film.

82

The wavenumbers pertaining to their corresponding functional groups are shown in Table 10.

Table (10). Functional groups and corresponding wavenumbers in FTIR

Group	Range cm^{-1}
-CH$_3$, -CH$_2$	2850 - 3000
=CH$_2$	3020 - 3100
-C=C-	1900 - 2000
-OH (free)	3580 – 3650
-OH (H is bonded)	3200 - 3550
-C=O (saturated aldehyde)	1720 - 1740

It can be observed from the Figures (29 - 32) of the untreated and treated films that they all possess a peak at 1410 cm^{-1} that corresponds to C-H aromatic ring vibration (reference band for PET) and a peak at 1340.6 cm^{-1} that corresponds to the trans-conformation of oxygen at the glycol segment and the trans-planar conformation of the terephthalate residue at the repeating unit of the polymer. These findings are the same as those reported in a study carried out by Pinto et al. (1999) who also proved that chemical modification produces a spectrum similar to that of the untreated film. It is also clear that the chemically modified film has a peak at 3210.8 cm^{-1} which corresponds to OH groups (with H that is bonded) of higher intensity than that of the untreated films which is absent in the other two film treatments. This peak is due to the high concentration of OH groups formed upon treatment due to the acrylic chemical that is added to the film. Another similar finding was reported by Song et al. (2006) who reported the presence of a peak at 3430 cm^{-1} that corresponds to free OH group. The peak intensity ratio between this peak and the one at 3054.6 cm^{-1} (corresponding to =CH$_2$ group) is

83

maximum in the chemically modified films and comparable for the other two treatments and the untreated film. Similar groups in all spectra include a peak at about 1732.1 cm^{-1} that corresponds to C=O group and peaks between 2900-2980 cm^{-1} that correspond to –CH$_2$ groups. Also, a peak is found at about 1960 cm^{-1} that corresponds to a C=C asymmetric stretch bond.

5.7. XPS analysis

Table 11 shows the XPS results pertaining to the untreated and treated films.

Table (11). XPS results for the chemically modified, plasma treated, corona treated films against the untreated film. (Data refer to the carbon and oxygen on the film surface)

	Peak	Position eV	Atomic concentration %
Untreated film	C 1s	284.96	72.31
	O 1s	531.86	27.69
Corona treated films	C 1s	284.96	71.22
	O 1s	533.45	28.78
Plasma treated films	C 1s	284.86	70.43
	O 1s	533.56	29.57
Chemically modified films	C 1s	284.99	68.78
	O 1s	533.49	31.22

The table shows that chemically modified films have the highest concentration of oxygen groups indicating that it is more hydrophilic than the other two types of treatments. This finding is in agreement with both water contact angle measurements and SEM micrographs. However, it can be deduced from the table that the plasma treated films are slightly more hydrophilic than the corona treated ones. This contradicts the contact angle and SEM results. Such a discrepancy could be owed to film ageing and surface contamination with time. According to Song et al. (2006) who studied the effect of grafting of acrylic acid onto PET on pressure-sensitive adhesive, the peak at 531 eV is assigned to -OH group which corresponds to

untreated films in the present work. However, the peak at position 533 eV is assigned to –C=O and –C-O- groups which are found in all the treated films studied in the present work. The highest % atomic concentration corresponding to this peak belongs to the chemically modified film confirming the grafting of the acrylic chemical onto the film. The peak at 284 eV is assigned to –C-C- and –C-H- bonds. This peak was found in the present work at its maximum value in the control and at its minimum value in chemically modified films.

5.8. Oxygen permeability test

The oxygen permeability test was performed on the chemically modified film only because this type of treatment has the highest hydrophilicity and will therefore be a good indicative of permeability. Permeability was estimated to be 160 ±6 ml $O_2/m^2/24$ hr/atm at 23 ºC. This value lies within the normal reported range for untreated PET films with a thickness of 12 μm (Ref: Dupont Teijin films: Product information). Thus, it can be concluded that chemical modification did not have an effect on the barrier properties of the films.

6. Conclusion

Corona treated, plasma treated and chemically modified PET films were coated with rosemary and clove extracts. DPPH scavenging activity and TBA antioxidant activity measurements showed that the coated films have potent antioxidant activities. Chemically modified films had the best adhesion properties among other films as indicated by DPPH scavenging activities, water contact angle and SEM microscopy studies. Although, corona and plasma treated films have higher tendency to release the coating material as compared to the chemically modified film, however they are not preferred in case of coating volatile substances as they do not have the capacity to retain these substances on the film surface during preparation. Furthermore, TBA results revealed that the three treatments do not show significant differences in protecting fish from oxidation. It can be also concluded that surface treated films coated with either rosemary or clove have a considerable potential to preserve fish from oxidation. Finally, plasma treated films should be last chosen for preparing antioxidant films for their poor adhesion while chemically modified films will be the best choice giving the best adhesion.

7. Future work

Our project can be extended to studying the antioxidant activity of other natural extracts coated with the same technologies. We can also compare the surface energy of chemically modified films to films with nano-coating. Another idea could be studying the antimicrobial properties of presently use or other extracts. Furthermore, this type of coating technology can be used in developing functional packaging achieved through coating bioactive molecules and testing them.

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