

ACTIVE AND INTELLIGENT PACKAGING: SMART BIOFILM WITH PLANT EXTRACT AS A POTENTIAL INDICATOR.

Submitted in partial fulfillment of the requirements for the award of bachelors of
Technology degree in Biotechnology

By

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**INSTITUTE OF SCIENCE AND TECHNOLOGY
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **L. Harinakshi (38230015)** who carried out the project entitled “**ACTIVE AND INTELLIGENT PACKAGING: SMART BIOFILM WITH PLANT EXTRACT AS A POTENTIAL INDICATOR**” under our supervision from September 2021 to April 2022.

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ABSTRACT

Active packaging is one of the interesting concepts in food industry which extend the shelf-life of the food products. The development of intelligent packaging based on natural and biodegradable resources is getting more attention by researchers in recent years. pH Intelligence packages can monitor the conditions of the food in the real time. The change in pH are indicative of food deterioration and can easily visualize by the consumer. pH monitoring is based on chitosan, PVA, EDTA, and plant extract. It is produced from medium molecular weight chitosan, PVA, EDTA, and phytochemical extract from plant. FT-IR, SEM, and Particle size are the characterisation test to analyze the biofilm. Color variations of pH indicator films were identified after immersion in different pH buffers. To validate the spoilage detection sensor, application test are conducted with meat or fruits. Finally it visually indicate the pH changes. Therefore, it has potential to be used as a visual indicator of the storage and consumption conditions of food and even it will increase the shelf life of food products.

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LIST OF SYMBOLS AND ABBREVIATIONS

HCL	Hydrochloric acid
SEM	Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
ZP	Zeta potential
EDTA	Ethylene diamine tetra acetic acid
NaOH	Sodium Hydroxide
PVA	Polyvinyl Alcohol
µg	Microgram
µl	Microlitre
ml	Millilitre

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CHAPTER 1

INTRODUCTION

1.1. OVERVIEW :

The food industry has invested in smart packaging to meet consumer demand for refreshing healthy products on a long shelf. Improving quality features in smart packaging has developments with pH indicators to promote the deliberate interaction of food. the use of these packages may enable the consumer to notice these changes in the diet, by changing the color of the package (Choi et al., 2017a). Intelligent packages can monitor food situations in real time. In order to reflect the quality of food to the consumer, it must be simple, sensitive and efficient. In many food products, changes in pH are an indication of food spoilage and can clearly show to the consumer any changes in food quality. Therefore, systems capable of monitoring, detecting, and visually detecting pH changes in food are improved, including indicators of pH changes in the product (Silva-Pereira et al., 2015) . Intelligent packaging, also known as smart packaging, is packaging in a way that recognizes some of the features of closed or stacked food and can inform the manufacturer, seller, and consumer of the status of these properties. Intelligent packaging can be established to hear, identify, record, and transmit information to assist in decision-making and to improve shelf life, to improve quality, to ensure safety(Ghoshal, 2018).). An effective nano-packaging system protects against microbial activity and acts as an oxygen and moisture barrier (Batool et al., 2021). In choosing the right food packaging, many variables such as ingredients, shelf life, availability and product compatibility should be considered. Globally, food security is a major public health problem. The main desire to ensure food safety by pleading to create packages that can prevent the entry of harmful food into the food, a great desire of consumers. Significantly, food must be stored in chemical, physical and natural contamination during preparation, storage and distribution (Singh et al., 2018). Typically, biopolymer-based insertion films are used as food wrappers, while edible coatings are applied directly to the food surface. Biopolymer-based packaging films and edible coatings combined with natural materials that can regenerate can effectively

restore oxidation and delay microbial degradation. Thus, the development of effective insertion films and edible coatings based on biopolymers and natural ingredients that work with healthy discovery has received increasing attention in recent years (Yong and Liu, 2021)

1.2 ACTIVE PACKAGING:

Active packaging refers to the installation of certain additives in packaging systems (whether open inside the package, attached to the inside of the packaging or inserted into the packaging itself) for the purpose of maintaining or extending product quality and shelf-life. The principles in active packaging are based on the internal properties of the polymer which are used as the original packaging materials or in the presentation of certain materials within the polymer (New, 2014). Active packaging, where packaging inputs does more work than basic content and protection, remains the site of active research with great potential for commercial applications. Active packaging can be applied to the packaging of food, pharmaceutical, and consumer goods products, with the sole purpose of improving the shelf life, safety, or quality of packaged goods. Synthesis can be achieved by inserting an active agent (e.g., antioxidant, enzyme, antimicrobial, oxygen scavenger) into or into the contact area of the packaging product (Bastarrachea et al., 2015). Active packaging systems should be considered an innovative way in the field of food packaging. They interact with the packed product, change the conditions of the packed food, and control its quality at the same time (Han JH et al., 2005). Active packaging represents a large and diverse group in terms of both its purpose and solutions applied. The use of proper active packing extends the shelf life of products through its impact on processes emerging in food like physiological processes, e.g., breathing of fresh fruit and vegetables; chemical processes, e.g., oxidation of fats; physical processes in the case of bread staling; microbiological changes due to the impact of microorganisms; infections caused by insects (Janicki A et al., 2013).

1.3 DEVELOPMENTS IN BIO-BASED SENSORS:

The development of bio-designed sensors (e.g., sensors that use compounds derived from natural products and the use of biopolymers) to be used as smart packaging for food products, focuses mainly on indicators. Freshness indicators are used in smart packaging to provide information on food quality decay. Natural pigments extracted from plants and fruits are regenerated, showing low toxicity, and minor environmental impacts. To be used as a pH indicator it is necessary to check the stability and pH response of pigments, which depends on each (Rodrigues et al., 2021). Bio-based sensors have at least one object from a bio-based source that may be a substrate (e.g., plastics listed in the previous section) or a sensor. Most of the bio-related sensors in food intake are based on the discovery of colorimetric analytics. Although colorimetric pH-sensitive sensors are generally not suitable for selective processing, it is usually sufficient to assess the quality of food in terms of pH changes, as degraded proteins produce alkaline chemical compounds of nitrogen (cadaverine, putrescine, histamine, and ammonia). Many fruits, berries, vegetables and flowers that cover almost everything visible are dyed with natural chemicals such as anthocyanins and curcumin known as natural pH indicators (Halonen et al., 2020)

1.4 ANTHOCYANINS USED AS BIO-BASED SENSORS:

Anthocyanins, a group of flavonoids most commonly found in natural products and known for their pH-sensitive color change, were chosen as an indicator solution for the formation of the reference sensor (Shukla et al., 2016). There are six types of anthocyanidins found mainly in nature: cyanidin, pelargonidin, delphinidin, petunidin, peonidin, and malvidin. Anthocyanins are found mainly in high plants and are not found in low plants except for other moss and ferns. Variable colors such as carmine, indigo blue, magenta, purple and pink found in various flowers,

fruits and vegetables are due to anthocyanins, and anthocyanin properties that change color depending on pH-change are well known(Roy and Rhim, 2021). Among plant-based dyes, anthocyanins have 65 sensitivity color responses to wide pH areas. Although anthocyanins extracted from 68 purple sweet potatoes have a higher resistance to heat and light, compared to 69 dyes extracted from other plants, the use of 70 red and white energy (PSPE) as a pH indicator paint has not been studied (Choi et al., 2017b). Anthocyanin-based compound has been successfully developed using chitosan and cellulose for the purpose of monitoring the deterioration of milk without the use of complex metals(Ebrahimi Tirtashi et al., 2019). Anthocyanin also has many health benefits, for example, antioxidant, antiinfigueatory, anticancer, antimicrobial and neuroprotective activities. Anthocyanins show color variation under different pH conditions. As the process of food spoilage is often accompanied by changes in pH, so anthocyanin-rich films can be used as clever pH indicators to show certain color changes and monitor food ripening (Xue Mei et al., 2020).

1.5 MOISTURE REGULATORS:

Food from moisture damage needs to be included in substances that prevent high humidity. A certain amount of moisture, however, can be trapped in the packets or accumulate during distribution. If not removed, this moisture will be absorbed by the product or condensate will form, causing bacterial damage and / or low consumer complaint. The main purpose of humidity control is to reduce aw, thereby reducing mold growth, yeast and bacterial damage in high-aw foods, such as ready-to-eat foods (Vermeiren et al., 1999). Moisture-absorbing pipes, sheets and blankets are used to control liquids in foods such as fish, meat, poultry, fruits and vegetables. Sheets and large sheets are used to absorb melted ice during the transport of cold fish goods(Biji et al., 2015).

1.6 ETHYLENE REMOVAL SYSTEMS:

The ethylene removal method requires, in some cases, additional treatment to reduce the rate of ethylene production (Stow, 1986). Ethylene removal package is a type of AP that intentionally and forcefully converts ethylene levels into a package through invasive chemicals. Chemicals such as potassium permanganate (KMnO_4), activated carbon, zeolites, and metal-organic compounds (MOFs) have been reported to be effective in removing ethylene (Awalgaonkar, 2018). Ethylene removal has been widely used as an additive procedure in modified or controlled storage, making testing of its direct effects on post-harvest fruit health difficult (Zamorano et al., 1994). Plants that have many effects on the growth, growth and maintenance of many fruits, vegetables, and ornamental plants. Its sources can include nearby plants, automotive extraction, plastics, smoke, fluorescent lamps. One of the easiest ways to prevent the harmful influence of ethylene on decaying plants, and thus to increase their shelf life, remove it from storage and management. Removing ethylene from the atmosphere surrounding new sensitive ethylene products is one of the major challenges in post-harvest rotting products to increase its youthfulness, quality, and shelf life from field to table (Keller et al., 2013)

1.7 ETHYLENE ADSORBENTS:

Adsorption is a ground substance where a particle is trapped in the surface of a solid object. In contrast, absorption is a mass in which particles are stored inside a solid substance. Active carbon / coal, zeolites and metal-organic structures can be absorbed / absorbed ethylene and as a result, are used to improve ethylene removal (Awalgaonkar, 2018). ethylene from the fruit environment by absorbing and synthesizing it to produce CO_2 and H_2O (Sharma and Sharma, 2016). Ethylene absorbent (EA) treatment has shown good ability to maintain fruit quality (Wang et al., 2018). The latest innovation is the use of

economically based KMnO₄ made of sacks placed inside packing boxes while transporting the 'Silver Queen' nectar (Jayarajan and Sharma, 2018).

1.8 ETHYLENE SCAVENGER:

Ethylene scavenger films, polymer films and polymer nanocomposite can be packaged as effective packaging to enhance product-shelf life (Srithammaraj, Magaraphan and Manuspiya, 2018). The effective ethylene absorbers can be divided into two main categories: ethylene absorbers and scavengers; absorbers are those substances that absorb and absorb ethylene molecule from the environment, and on the other hand, ethylene scavenger, where water absorption is due to a chemical reaction between two substances. ethylene scavengers in the form of powder, granules, and beads, are usually placed in a bag made of material (Gaikwad, Singh and Negi, 2020).). Control of ethylene in stored conditions plays a major role in enhancing the post-harvest life of many new product types. Ethylene scavengers are useful for storing ethylene-sensitive fruits and vegetables such as apples, bananas, mangos, tomatoes, onions, carrots(New, 2014) Packing ethylene brushes, such as zeolite, titanium dioxide and flexible metals, in a small bag was used almost and was widely reported. Ethylene (C₂H₄) acts as a plant hormone that has different physiological effects on fresh fruit and vegetables. It accelerates respiration, leading to maturity and senescence, and also softening and ripening of many kinds of fruit. Furthermore, ethylene accumulation can cause yellowing of green vegetables and may be responsible for a number of specific postharvest disorders in fresh fruits and vegetables(Vermeiren *et al.*, 1999). Ethylene capsules can be embedded firmly, dispersed in plastic, or packaged in various parts of the package. However, the inclusion of ethylene scavenger in food packaging or in films and in-situ oxidation of ethylene is rarely reviewed. (Wei *et al.*, 2021).

1.9 CARBON DIOXIDE SCAVENGERS:

CO₂ scavengers of food packages is beneficial in maintaining food quality and package

integrity (Lee, 2016). Many sachet and label resources can be used to manipulate or remove carbon dioxide (Day, 2008) carbon dioxide scavengers reduce the increase in gas pressure within solid packaging, or volume expansion in flexible packaging by absorbing carbon dioxide produced by respiration (Aday, Caner and Rahvali, 2011). CO₂ is formed in some foods due to decomposition and respiratory response. The CO₂ produced should be removed from the package to avoid food spoilage and / or package destruction (Vermeiren *et al.*, 1999).

1.10 ANTIMICROBIAL ACTIVE PACKAGING:

To control minor foodborne illness, antimicrobials can be packaged or packaged in food packaging. The principle action of antimicrobial films depends on the release of antimicrobial organizations, some of which may pose a safety risk to consumers if the release is not strictly regulated by other means within the packaging material (Vermeiren *et al.*, 1999). Antimicrobial packaging is related to improving food safety. This type of packing slows down the growth of bacteria or reduces the growth rate and the final number of germs. The most popular antimicrobials for active packaging include nisin, chitosan, potassium sorbate, silver-based zeolite, and essential oils (Dobrucka and Przekop, 2019). Many challenges, including the controlled release of antimicrobial agents and the manufacture of packaging materials (especially bio-based materials) with adequate barrier properties, transparency, durability and other factors to meet food safety and food safety requirements, remain to be addressed in these new effective packaging methods. antimicrobial. Depending on the type of antimicrobial agent, they can cause significant changes in the complex properties of packaging materials, including their strength, strength, consistency, and material and thermal properties even in appearance (Mousavi Khaneghah, Hashemi and Limbo, 2018).

1.11 CARBON DIOXIDE:

Carbon dioxide is a colorless gas, and is often used in food products (e.g., carbonated beverages) to provide the consumer with the desired properties (Dobrucka and

Przekop, 2019). Carbon dioxide suppresses microbial activity. Extremely high levels of CO₂ (60 to 80%) inhibit bacterial growth in the surface areas and, in addition, increase the shelf life of packaged food (New, 2014). CO₂ packaging can damage product quality and / or package integrity (Lee, 2016). CO₂ efficiency is mediated by storage temperature, and storage with lower temperatures leads to better control of microbial growth (Lee et al., 2015).

1.12 ETHANOL:

Spraying ethanol in food product areas, such as bread, cookies, and other bakery products, has been shown to be effective in prolonging the shelf life of these substances by suppressing fungal growth. The effectiveness of the ethanol production system depends largely on the type and size of the container, the amount of ethanol held by the carrier, sachet material in water vapor and ethanol, food water function, and ethanol penetration of the packaging film (Ozdemir and Floros, 2004). Ethanol vapor also has an anti-additive effect on top of its antimold properties. A bag called Ethicap® produces ethanol smoke and can be used for cakes and breads (Patel, Prajapati and Balakrishnan, 2015). Ethanol-producing technology was originally developed in Japan where the ethanol intake range was coated with fine inert powder inside the bag (Mane, 2016).

1.13 PRESERVATIVES:

Preservative supplements are substances used to prevent unpleasant changes in diet during storage and travel. Preservatives are divided into three groups; antimicrobials, antioxidants, and antibrowning agents. Natural remedies and spices have the function of preserving the material depending on the type, nature and concentration of the body. Herbs and spices have a wide range of chemicals. These chemicals are extracted in a variety of ways and are used in conservative production (Gokoglu, 2019) Food preservation is an ingredient used to save food by lowering the pH value and resolving the ability to eat more (Sharif et al.,

2017). Varieties of plant preservatives found in plants (e.g., essential oils and plant extracts), animals (e.g., chitosan and lysozyme), or microorganisms (e.g., bacteriocin and organic acids) have been applied to many food products (Hassoun *et al.*, 2020). The extraction of plants and essential oils has been unique in the production and storage of chemicals, especially to provide environmental protection without spoilage and extend shelf life in animal feed (Erkan, 2015)

1.14 INORGANIC NANOPARTICLES:

Inorganic nanoparticles such as zinc oxide (ZnO), silver (Ag), titanium dioxide (TiO₂), silicon dioxide (SiO₂), and iron (III) oxide (Fe₃O₄) have been used as metals. -cross-link agents to improve mechanical, physicochemical, water-related, and antimicrobial properties in polysaccharide-based foods for active packaging. The use of rare nanoparticles has exploded in recent years, using it in a variety of fields, such as textiles, cosmetics, optics, agriculture, pharmaceuticals, pharmaceuticals, and food packaging (Anaya-Esparza *et al.*, 2021). Inorganic nanoparticles are known to be among the most widely used antibacterial agents in various fields, especially in the food packaging industry. inorganic nanoparticles antimicrobial activity can include a large group of microorganisms including food-borne pathogens (Hoseinnejad, Jafari and Katouzian, 2018).

1.15 PHYTOCHEMICALS:

Phytochemicals, commonly referred to as phytonutrients, are natural components of foods rich in foods such as vegetables, fruits, whole grain products, nuts and seeds, legumes, tea and dark chocolate. The most common phytochemicals in the diet include polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfures, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidin, phenylpropanoids, anthraquinones, ginsenosia, ginsenosia, and so on (Xiao, 2016). Phytochemicals are small molecules that are commonly used by humans and play an important role in

improving the health of patients suffering from various diseases(Xu and Lu, 2019)

1.16 INTELLIGENT PACKAGING:

Intelligent packaging is defined as a sensor that is responsible for hearing the environment and restaurants and processing details. There are several types of smart packaging: indicators, including temperature indicators (TTIs), gas sensors, maturity indicators, toxicity and biosensor indicators, and radio frequency (RFID) detection that directly measures product quality within the package (Lee et al., 2015). Intelligent packaging is a solution based on a partnership between packaging and food or environmental products, its function is to inform the manufacturer and consumer about product renewal. Intelligent packaging monitors and alerts customers to product innovation throughout the “product life.” (Dobrucka and Przekop, 2019). Approval of Effective and Smart Packing (ACTIPAK-FAIR CT 98-4170, 1999-22001) as 'integrated food monitoring systems to provide information on the quality of integrated food during transport and storage'(Kerry, O'Grady and Hogan, 2006). Intelligent packaging (also described as smart packaging) is packaging that in some way senses some properties of the food it encloses or the environment in which it is kept and which is able to inform the manufacturer, retailer and consumer of the state of these properties. Although distinctly different from the concept of active packaging, features of intelligent packaging can be used to check the effectiveness and integrity of active packaging system(Jaiswal et al., 2013).

1.17 INDICATORS:

An indicator can be defined as a substance that indicate, presence or absence of an object or the degree of reaction between two or more objects through the change of character, especially in color(Kerry, O'Grady and Hogan, 2006).). Indicators are called intelligent or interactive because they interact with compounds in food (Dobrucka and Przekop, 2019). Indicators should be simplified

and reflect changes (or indications) that are easily measurable and irreversible, time and temperature-dependent changes must be reproduced and adapted accordingly or easily to food quality, and provide package information(Molga, 2014).). Various types of indicators are used, for example, non- perishable biological materials, temperature indicators (TTIs), corrosion, chemicals, or other pollution indicators(Ghoshal, 2018).

1.18 SENSORS:

A sensor is defined as a device used to detect, detect or measure power or matter, providing a signal for the detection or measurement of a physical or chemical substance to which a device responds. Many intelligent packaging concepts include the use of sensors and indicators. The development of improved methods of determining food quality such as fresh, antibacterial, oxidative or oxygen depletion and / or heat-induced degradation is particularly important for food producers(Kerry, O'Grady and Hogan, 2006).

1.19 BIOSENSORS:

Biosensors are integrated analytics devices that detect, record and transmit information related to biological responses. Intelligent packaging systems that include biosensors have the potential for extreme precision and reliability (Kerry, O'Grady and Hogan, 2006). Biosensors may allow remote monitoring of package conditions or point test status of the product in conjunction with appropriate electrical equipment. An effective pattern for the use of non-respiratory bioactive chemicals in intelligent packaging units obtained from biosensors (Ghoshal, 2018).

1.20 EDIBLE SENSORS:

The use of edible film sensor as food packaging material is expected to reduce plastic waste. Edible films should contain substances produced mainly by edible biopolymers and dietary supplement (Dinika and Utama, 2019). Food packaging has been

researched as an effective way to improve food quality. Edible coverings and films can often be defined as small layers of food used in or within food by dipping, brushing, spraying or wrapping (Erkan, 2015). Consumers seem willing to accept other products that use edible, perishable, and friendly packaging (Hassoun et al., 2020). It can provide antioxidant, antioxidant, and antimicrobial binding foods, thereby significantly reducing the incidence of disease while extending the shelf life of the food (Yong and Liu, 2021).

1.21 SPECIFIC CHEMICALS AND PH CHANGES:

The inclusion of anthocyanins, which are widely available as antioxidants and pigments in plants, is actively indicating differences in pH values near the interaction between feed and package. The anthocyanins found in red cabbage can vary in color from red to purple and blue with different pH values, which facilitates the acceptance of food quality depending on the pH change. At pH 1, it shows a red color, and it dramatically changes its color from red to purple as the pH is raised to 6. It is blue at pH 7 and 8, but changes to sea green at pH 9. With an increase in pH to 12, the film quickly turns green (Vo and Dang, 2019).

1.22 BIOSENSORS FOR BACTERIA DETECTION:

A biosensor is a scientific tool that combines a biomolecule to find and perhaps measure an analyte. Biosensors can be used to detect pathogenic bacteria in a fast and inexpensive way. Complete bacterial biosensors can also find dead cells, but they generally depend on the integrity of the cell structure of the biomolecule which can also degrade immediately after cell death. The most common way to detect the attachment of targeted microbes is to use a biomolecule, after which it is concentrated near or in a transducer (Burlage and Tillmann, 2017). Biological detection biosensors usually involve a biological recognition component such as receptors, nucleic acids, or antibodies in close contact with the appropriate transducer. Bacterial biosensors can also be divided into batch (intermittent) sensors and continuous (monitoring) mode.

Bacterial biosensors should reduce human participation (to avoid contamination) and therefore, automation should be the biosensor's responsibility(Ivnitski et al., 1999). Biosensors act based on the acquisition of a substrate or enzyme reaction product(Bahadir and Sezgintürk, 2016)

CHAPTER 2

REVIEW OF LITERATURE

In the USA, Japan and Australia, practical packaging concepts are already being used successfully. In Europe, the development and use of functional packages is limited due to legal restrictions, fear of consumer resistance, lack of operational knowledge, economic and environmental impact Vermeiren et al., 1999.

Active packaging is an emerging field of food technology that can offer many savings benefits to many types of food products. The main purpose of effective packaging systems is to maintain sensory quality and extend the shelf life of the food while maintaining the quality of healthy food and ensuring the safety of bacteria. This results in a decrease in food waste at the same time Melini and Melini, 2018.

The effective use of nanocolloidal particles in various branches in the food industry, such as food quality, safety, nutrition, processing, and packaging, has been widely reported recently Nile et al., 2020.

Smart nanomaterials offer the potential for many disruptive technologies to be widely used in the future through the food packaging application Jiang et al., 2015.

One of the newest and most prominent trends in AP is the aging package of multi-functional performance. A combination of effective and intelligent packaging is the closest packaging technology. Some authors have described this method of technology as 'intelligent (intelligent) packaging'. It is noteworthy that effective and efficient installation is very important in industry 4.0. This simplifies production processes, facilitates the distribution of goods, protects and improves product quality, and provides product information during marketing, among others Alonso et al., 2021.

Haghighi et al., 2019 explained that the recent sharp rise in exposure to environmental concern arising from plastic packaging has sparked interest in more environmentally friendly packaging materials. This latest trend encourages the commercialization of information through the use of chitosan-based films. Because of its unusual biological

and functional properties, chitosan has been extensively researched and used. However, inherent flaws such as a low mechanical properties and high susceptibility to humidity restrict its industrial applications, which include food packaging. The scientific literature addressing chitosan-based films for their potential application in the food packaging industry has been extensively reviewed in the current research.

Alizadeh-Sani et al.,2020 clarified that the new ascent in familiarity with safe food and changing buyer perspectives has acquired development bundling innovation. Customers are progressively requesting common food colorants like carotenoids, betadine, anthocyanins, and chlorophylls instead of engineered colors for food applications. Accordingly, shrewd bundling dependent on characteristic colorants and biopolymers has been presented as the most recent innovation in the food bundling field. Keen items shield food from natural risks, yet additionally convey ongoing messages (colorimetric, synthetic, or electrical) to shoppers for changes in the bundling climate and food quality.

Kerry, O'Grady and Hogan, 2006 An indicator may be defined as a substance that indicates, the presence or absence of another substance or the degree of reaction between two or more substances by means of a characteristic change, especially in colour. In contrast with sensors, indicators do not comprise receptor and transducer components and communicate information through direct visual change.

Biji et al., 2015 According to EC/450/2009, intelligent materials and articles means materials and articles which monitor the condition of packaged food or the environment surrounding the food. Intelligent packaging systems provide the user with information on the conditions of the food or its environment (temperature, pH).It is an extension of the communication function of traditional packaging and communicates to the consumer based on its ability to detect, sense and record the changes in the products environment.

Lee et al. 2008 developed a new range of colorimetric oxygen indicators that are irreversible, reusable, and UV-light activated. Such “intelligent ink” oxygen sensors comprise a UV-absorbing semiconductor, such as TiO_2 , a redox-indicator, such as methylene blue, a sacrificial electron donor, such as triethanolamine, and an encapsulating polymer such as hydroxyethyl cellulose; the ingredients are mixed together, with water as the solvent, to form an ink. The ink can be coated or printed subsequently onto a variety of substrates to produce a blue oxygen indicator film, which, when activated by UV light, becomes colourless.

CHAPTER 3

AIM AND OBJECTIVE

3.1 AIM:

To develop a smart biofilm with plant extract as a indicator for active and intelligent packaging.

3.2 OBJECTIVES:

- To develop an cost effective Active pH and Intelligent smart packaging as a quality indicator in packed (Exported) food product.
- Smart packaging works both as a quality indicator and also increases the shelf life of the food material with the biopolymer of a plant extract present in the wrap.
- The present work will make a new stride in the food packaging sector by the development of this economically affordable and effective material.

CHAPTER 4

MATERIALS AND METHODS

4.1.SAMPLE COLLECTION

Sample of blueberry (Fig 1) are collected from the nearby fruits shop from mogappair in Chennai district, Tamil Nadu and washed thoroughly with water and then it was crushed and macerated.

4.2. EXTRACT PREPARATION

A sample of approximately 375g of blueberry was crushed and macerated with 80ml of ethanol - water (7:3). The pH of the sample was adjusted to 2.0 with HCl (1 mol/L). Subsequently, the material was stored for 24hr at 5°C, protected from light. After this duration, the material was filtered and the extract was centrifuged at 103×g for 10min. The supernatant was filtered on Whatman paper#1 and the resulting extract was neutralized to pH 7.0 with NaOH 2.5 mol/L.(Fig 2)(Silva-Pereira et al.,2015).

4.3. PREPARATION OF CHITOSAN, EDTA AND PVA FILM FORMING SOLUTIONS:

The 2% w/v chitosan (CS) solution was made by dissolving CS and EDTA at a 2:1 weight ratio in distilled water.

The PVA solution (10% w/v) was made by dissolving PVA in distilled water at 80°C and stirring for 4 hours. At a weight ratio of 30/70, the CS EDTA solution was combined with a PVA solution. The 30/70 CS EDTA/PVA solution was agitated for 24 hours after blueberry extracts (containing 1, 2 and 3 percent wt-blueberry to polymer) were added. The solutions' viscosity, conductivity, and surface tension were all measured. (Charernsriwilaiwat et al., 2013)

4.4. pH ANALYSIS FOR BLUEBERRY EXTRACT:

Take 2ml of blueberry extract in 14 tubes. The extract was adjusted accordingly with HCl(1 mol L⁻¹) and NaOH (1 mol L⁻¹) to pH values (1 to 14)(Fig 3).

4.5. PREPARATION OF pH INDICATOR FILM BY ELECTROSPINNING METHOD:

A 10 ml glass syringe with a 20gauge stainless steel needle (diameter = 0.9 mm) at the nozzle held the electrospinning fluid. The needle was attached to a Gamma High Voltage Research device's positive polarity emitting electrode. 5 kV was used as the electric potential. The nanofibers were gathered on an aluminium sheet wrapped around a spinning collector as they were spun. During spinning, a syringe pump was used to supply the fluid, and the rate was set at 0.25 ml/h. At room temperature, the solution was electrospun, and the collection distance was set at around 20 cm. The process time was set at 8 hours to produce mats with a thickness of 20-30mm.(Fig 4)(Charernsriwilaiwat et al., 2013)

4.6. FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR):

Fourier-transform infrared (FTIR) spectra were obtained using an FTIR spectrometer (Vertex 70/80, Bruker, Germany), operated in attenuated reflectance (ATR) FT mode. The films were measured at a resolution of 4 cm⁻¹ and in a wavenumber range 500–3500 cm⁻¹.

4.7. SCANNING ELECTRON MICROSCOPY (SEM):

Micrographs of the samples were acquired at room temperature using a scanning electron microscope (SU8010, Hitachi, Tokyo, Japan) with an accelerating voltage of 3.0 kV. The cross-sections and film surfaces were coated for observation.

4.8. ZETA POTENTIAL ANALYSIS:

The mean diameter and ZP of biofilm it is were determined using a dynamic light scattering (DLS) PS analyzer (ZetaSizer Nano ZS ZEN3600 from Malvern Instrument; Particle Sizing Systems, Inc. Santa Barbara, CA). The samples were diluted to 100-fold serial dilution) with deionized water. All measurements were carried out at 25°C, and the results were reported.

4.9. APPLICATION OF STRAWBERRY AS A DETERIORATION INDICATOR:

In order to evaluate the application of the film as strawberry deterioration indicator, strawberry acquired in local market were brought and wrapped with the pH indicator film and placed in a 60 mm culture plate. Two samples were kept under refrigeration temperature (4°C) and two under room temperature (25°C). The pH change in the headspace of the samples was monitored during 72 h, in order to evaluate colour change.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. SAMPLE COLLECTION:

Blueberry is collected from the nearby fruits shop from mogappair in Chennai district, TamilNadu.



Fig 1 – blueberry fruits

5.2. EXTRACT PREPARATION:

Blueberry is crushed and macerated using mortar and pestle. The extract is filtered using Whatman filter paper.

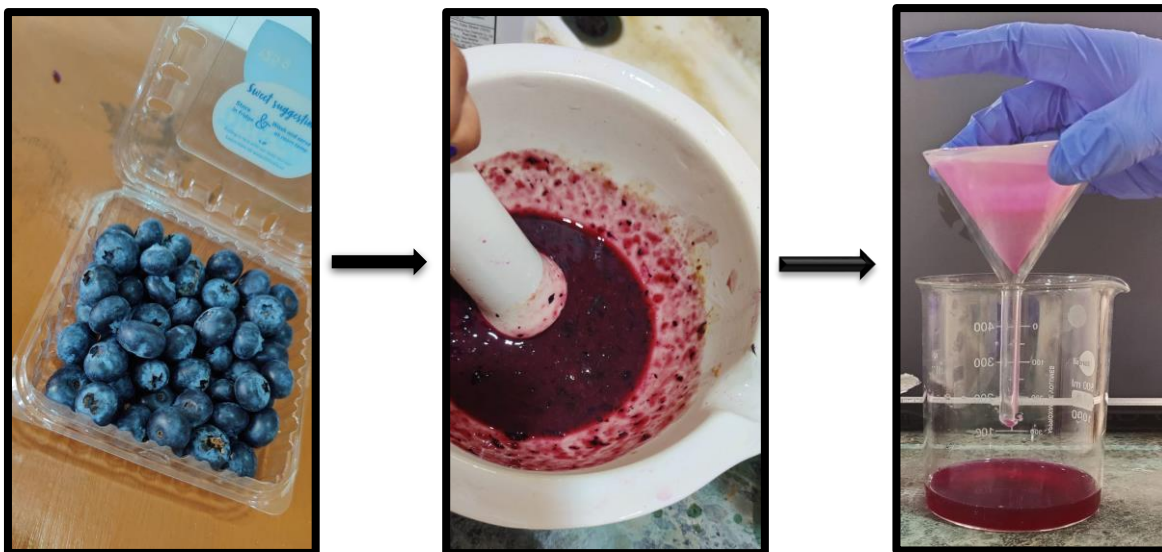


Fig 2 – blueberry extract is prepared by maceration method.

5.3. pH ANALYSIS OF BLUEBERRY EXTRACT:

Blueberry extract of 2ml is taken to analyse the pH. HCl and NaOH is added to the extract accordingly to adjust the pH. pH analysis of blueberry extract is done to obtain the colour variations in the biofilm.

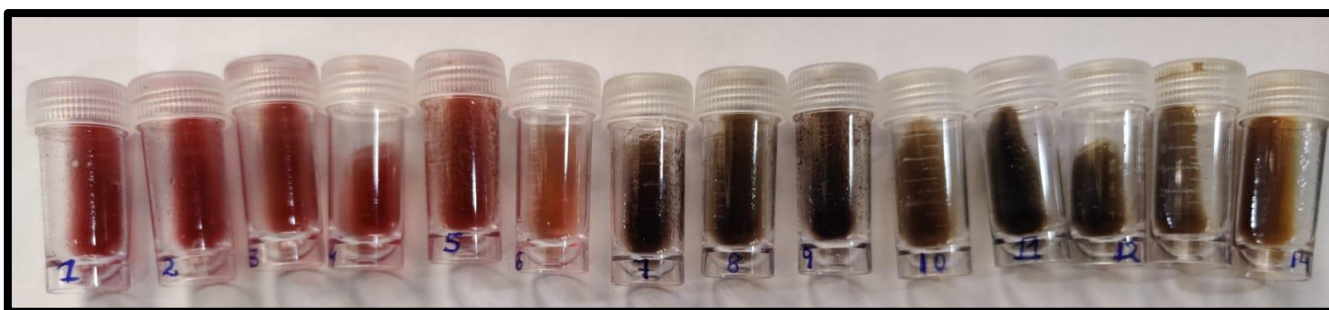


Fig 3 – pH analysis of blueberry extract from pH 1 – pH 14.

5.4. PREPARATION OF pH INDICATOR FILM BY ELECTROSPINNING METHOD:

In 10ml syringe the electrospinning fluid is added to it and they are allowed to run at the rate of 0.25ml/h. They are electrospun for 8 hours and the film is obtained at the thickness of 20-30mm.



a)



b)

Fig 4- a) Electrospinning method; b) Electrospinning process with the solutions.

5.5. FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR):

FT-IR spectroscopy was conducted to identify the functional group's present a sample of biofilm. By using FT-IR analysis, the infrared emission spectrum, absorption, photoconductivity can be determined. The FT-IR spectrum represents a character of the film consisting of absorption peaks that determine the frequencies of vibrations between the bonds of atoms of the sample. The highest peak position is 1085.54 cm^{-1} .

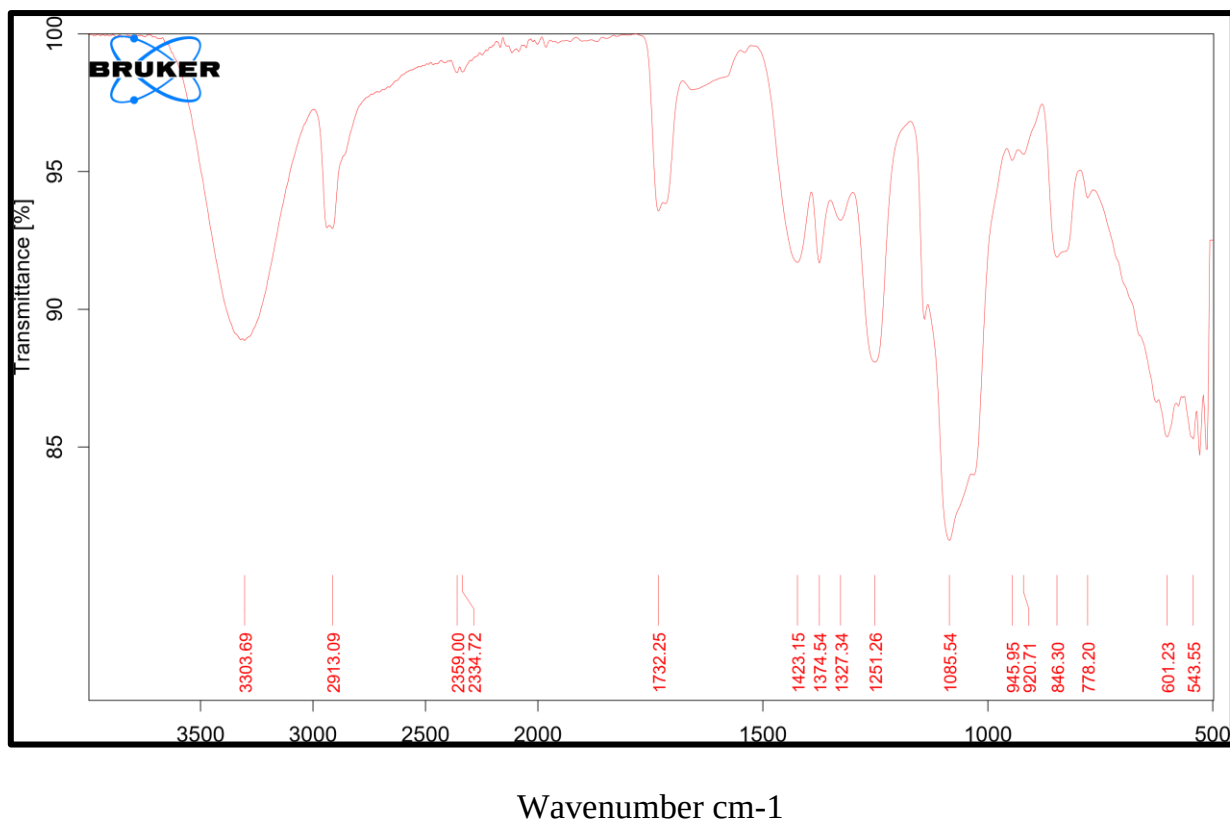
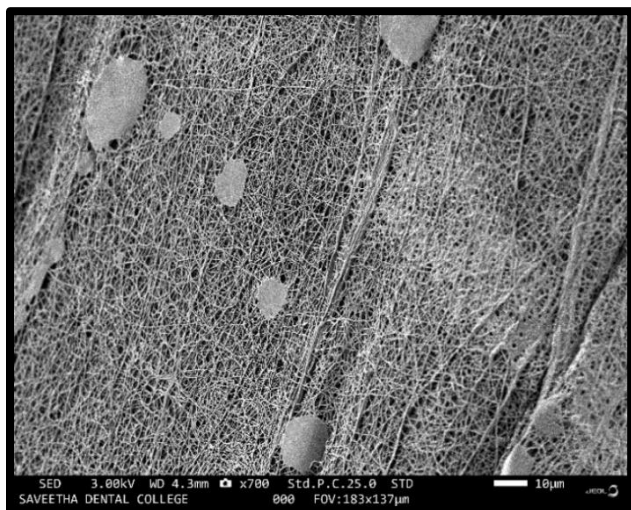


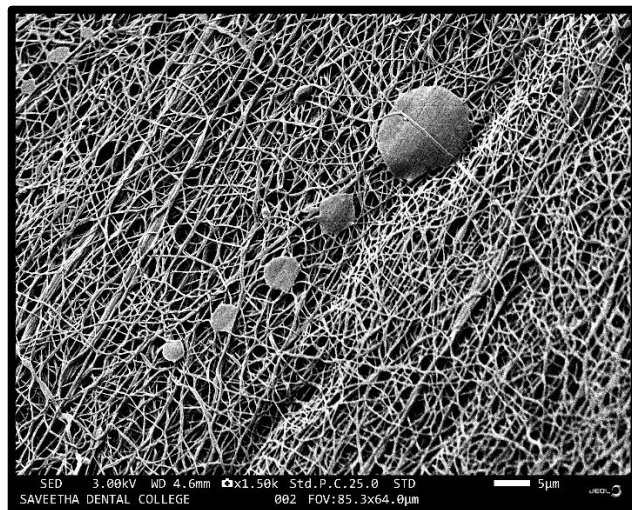
Fig 5 – FTIR Spectroscopy

5.7. SCANNING ELECTRON MICROSCOPY (SEM):

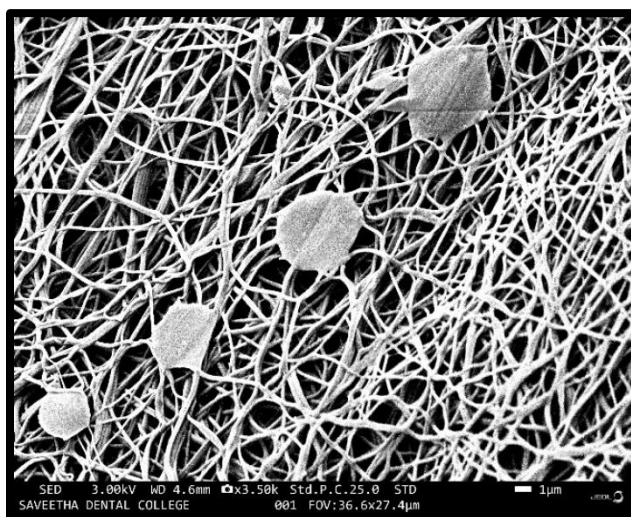
SEM analysis was employed to characterize the microstructure of particles, such as the size, shape, and dispersion patterns of synthesized biofilm. The SEM micrographs of biofilm are shown (Fig. 6). In this study the biofilm demonstrated fibre bodies with uniform nanostructure.



a)



b)



c)

Fig 6 (a,b,c) – SEM Micrographs

5.8 ENERGY DISPERSIVE X-RAY ANALYSIS (EDX):

In order to confirm the formation of biofilm EDX analysis was performed. During the EDX measurement area were focused and the corresponding peak is shown in (Fig:7).

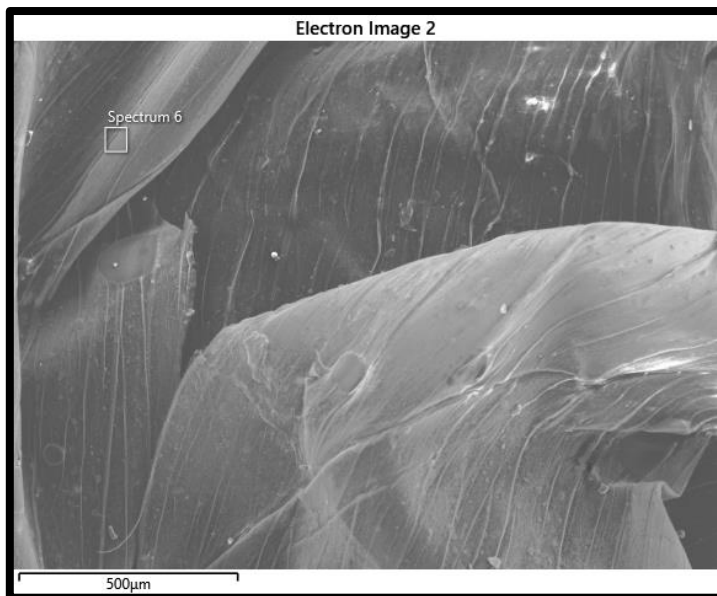


Fig 7 – electron image

	wt%	σ
C	61.2	0.2
O	37.6	0.2
Na	0.4	0
Cl	0.3	0
Cu	0.2	0
Zn	0.1	0
K	0.1	0
Si	0.1	0

Table 1 – spectrum values

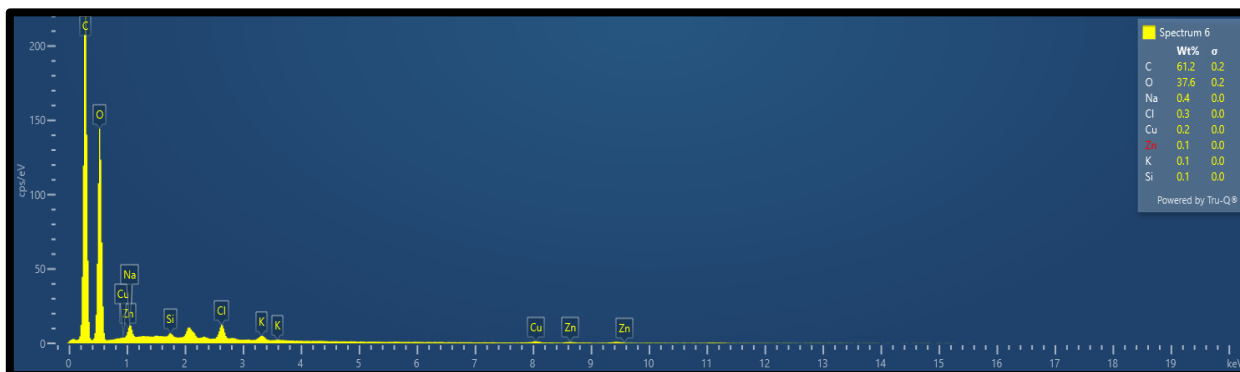


Fig 8 – spectrum graph

5.9. ZETA POTENTIAL ANALYSIS :

The surface charges an important criterion to determine the stability and conductivity of particle systems to biological surfaces. Therefore, the investigation of zeta potential a crucial component for the characterization of nanoparticles. The biofilm exhibited a positive zeta potential value, depending on its surface charge (Fig: 9)

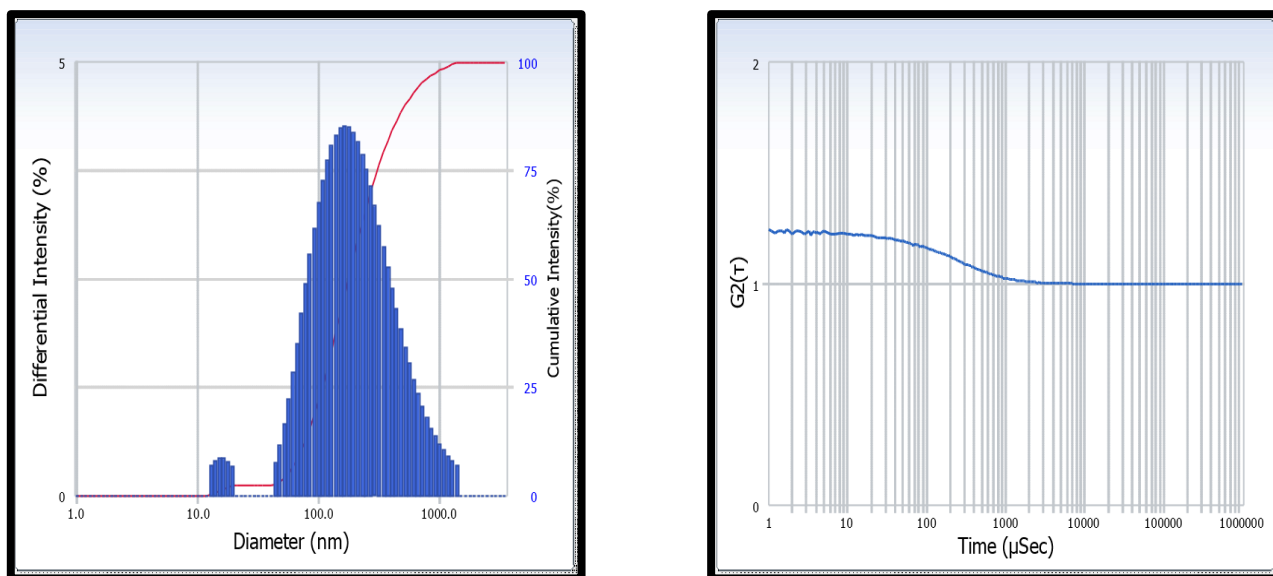


Fig 9 – zeta potential

Table 2 – zeta potential distribution results and cumulant results.

Distribution Results (Contin)		Cumulants Results	
		Diameter (d) :216.7	(nm)
		Polydispersity Index (P.I.) :0.220	
Peak	Diameter (nm)	Std. Dev.	
1	16.2	2.2	
2	275.9	240.0	
3	0.0	0.0	
4	0.0	0.0	
5	0.0	0.0	
Average	269.9	240.4	
Residual	: 7.714e-003	(O.K)	
		Diffusion Const. (D) :2.270e-008	(cm ² /sec)
		Measurement Condition	
		Temperature :25.0 Diluent Name :WATER	(°C)
		Refractive Index :1.3328	
		Viscosity :0.8878	(cP)
		Scattering Intensity :32706	(cps)
		Attenuator 1 :0.27	(%)

Table 3 – Intensity distribution table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
1.0	0.0	0.0	8.0	0.0	0.0	63.4	1.4	6.6	504.9	1.9	88.6
1.1	0.0	0.0	8.7	0.0	0.0	68.9	1.8	8.4	548.6	1.7	90.3
1.2	0.0	0.0	9.4	0.0	0.0	74.9	2.1	10.5	596.0	1.5	91.8
1.3	0.0	0.0	10.2	0.0	0.0	81.3	2.4	12.9	647.6	1.3	93.1
1.4	0.0	0.0	11.1	0.0	0.0	88.4	2.8	15.7	703.6	1.2	94.3
1.5	0.0	0.0	12.1	0.0	0.0	96.0	3.1	18.8	764.5	1.0	95.3
1.6	0.0	0.0	13.1	0.3	0.3	104.3	3.4	22.2	830.7	0.9	96.2
1.8	0.0	0.0	14.2	0.4	0.8	113.3	3.6	25.8	902.6	0.8	97.0
1.9	0.0	0.0	15.5	0.4	1.2	123.2	3.9	29.7	980.7	0.7	97.7
2.1	0.0	0.0	16.8	0.4	1.6	133.8	4.0	33.7	1065.5	0.6	98.3
2.3	0.0	0.0	18.3	0.4	2.0	145.4	4.2	37.9	1157.7	0.5	98.8
2.5	0.0	0.0	19.8	0.3	2.3	158.0	4.2	42.1	1257.9	0.5	99.2
2.7	0.0	0.0	21.6	0.0	2.3	171.6	4.3	46.4	1366.8	0.4	99.6
2.9	0.0	0.0	23.4	0.0	2.3	186.5	4.2	50.6	1485.0	0.4	100.0
3.2	0.0	0.0	25.4	0.0	2.3	202.6	4.2	54.8	1613.5	0.0	100.0
3.5	0.0	0.0	27.7	0.0	2.3	220.2	4.1	58.9	1753.2	0.0	100.0
3.8	0.0	0.0	30.0	0.0	2.3	239.2	3.9	62.8	1904.9	0.0	100.0
4.1	0.0	0.0	32.6	0.0	2.3	259.9	3.8	66.6	2069.7	0.0	100.0
4.5	0.0	0.0	35.5	0.0	2.3	282.4	3.6	70.1	2248.8	0.0	100.0
4.8	0.0	0.0	38.5	0.0	2.3	306.9	3.3	73.5	2443.4	0.0	100.0
5.3	0.0	0.0	41.9	0.0	2.3	333.4	3.1	76.6	2654.8	0.0	100.0
5.7	0.0	0.0	45.5	0.4	2.7	362.3	2.9	79.5	2884.5	0.0	100.0
6.2	0.0	0.0	49.4	0.6	3.3	393.6	2.6	82.1	3134.1	0.0	100.0
6.7	0.0	0.0	53.7	0.8	4.1	427.7	2.484.5		3405.3	0.0	100.0

d (nm)	f(%) f(cum.%)	d (nm)	f(%) f(cum.%)	d (nm)	f(%) f(cum.%)	d (nm)	f(%)f(cum.%)
7.3	0.0 0.0	58.4	1.1 5.2	464.7	2.2 86.6	3700.0	0.0 100.0
D (10%) :	73.40 (nm)	D (50%) :	184.30 (nm)	D (90%) :	541.00 (nm)		

5.13. APPLICATION OF STRAWBERRY AS A DETERIORATION INDICATOR:

At room (25°C) and refrigeration temperature (4°C), the indicator film (CSE). At room temperature, for 12 Hr no colour change was visible in the indicator film, after 16 h the colour began to change to blue, indicating pH increase and initial spoilage, and after 72 h the colour completely changed to yellow thus indicating complete spoilage. At refrigeration temperature, no colour change was observed during 72 h, but after this time, the colour changed to light blue and to yellow after 5 days, indicating spoilage. Therefore, the film can detect pH changes, which is an indicative of spoilage. Although other tests, such as microbial spoilage tests, are needed to validate spoilage and test the limitations of this system, it is accepted in the literature that pH changes in are a reliable indicator of its spoilage.

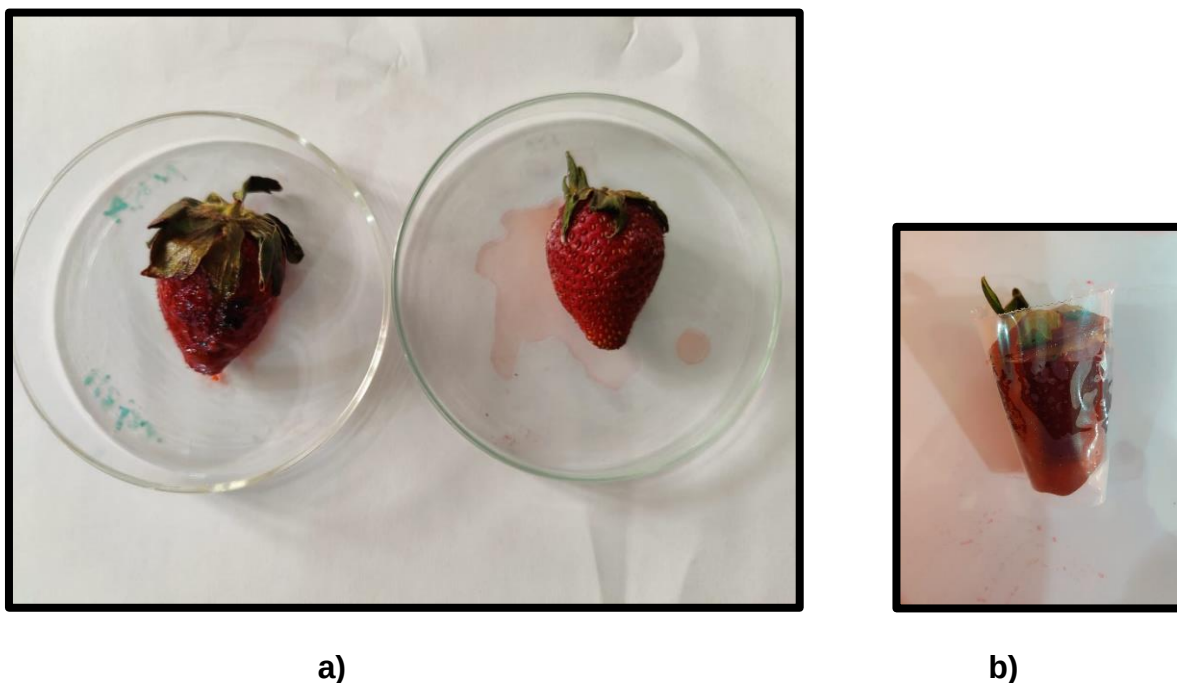


Fig 10 - a) visual observation of strawberry in room temperature ;b) strawberry wrapped with the film and kept in room temperature.



a)



b)

**Fig 11 - a) visual observation of strawberry in refrigeration temperature;
b) strawberry wrapped with the film.**

CHAPTER 6

SUMMARY AND CONCLUSION

The blueberry sample were collected from the fruits shop and extract is obtained by maceration method. Then blueberry extract and Chitosan, EDTA, PVA film forming solutions where incorporated and allowed to run in electrospinner. The indicator film also has good response to pH variation, safely detecting pH changes. Thus, it has potential to be used as a visual indicator of food deterioration. The contact between the polymer chains and the phenolic components of the extract kept the indicator films intact even at acidic pH, which was attributed to the interactions between the polymer chains and the phenolic components of the extract. Due to growing concerns about the safety of synthetic colourants, natural colourant sources have been examined. The active and intelligent packaging materials develop in the search of environment-friendly packaging solutions. In intelligent packaging systems they help to protect consumers from food borne illness. In upcoming years edible films gain increasing attention in food industry. Packaging with more functionality is continually being developed being investigated in response to consumer desire for less processed foods less preservatives in processed foods, and increased regulatory oversight market globalisation and needs In recent years, extensive research into the creation of novel active packaging technologies with natural pigments has resulted in a wide range of active packaging systems that can be used to increase the shelf life of food products.

CHAPTER 7

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