

CHARACTERIZATION AND APPLICATION OF EDIBLE FILMS BASED ON GELATINE AND COMPOSITE OF GELATINE, AGAR, & STARCH CONCENTRATE

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Technology degree in Chemical Engineering

BY

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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This is to certify that this Project Report is the bonafide work of **R. Selva kumar** (Reg.No. 39690018) who carried out this Project entitled "**CHARACTERIZATION AND APPLICATION OF EDIBLE FILMS BASED ON GELATINE AND COMPOSITE OF GELATINE, AGAR AND STARCH CONCENTRATE**" under my supervision from July 2022 to October 2022.

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DECLARATION

I, **R. Selva kumar (39690018)** hereby declare that this Project Report entitled **“CHARACTERIZATION AND APPLICATION OF EDIBLE FILMS BASED ON GELATINE AND COMPOSITE OF GELATINE, AGAR AND STARCH CONCENTRATE”** done by us under the guidance of **Mr. Michael Rahul Soosai (Assistant Professor, M.Tech., Ph. D)** is submitted in partial fulfillment of the requirements for the award of the Bachelor of Technology, degree in Chemical Engineering.

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ABSTRACT

The purpose of this work is to attempt to explain the usefulness of edible coating and films used in food packaging. We can realize how our earth is being harmed by the growing burden of non biodegradable materials in the environment. Thus, there is a urgent need to create edible packaging and films.

In this experiment, these natural degradable films were prepared from Gelatine as source ingredients and from composite of Gelatine, Agar, and Starch as source ingredients. Glycerol or Glycerin used as plasticizer is added to the source ingredients to increase its flexibility and Plasticity.

For Composite sample concentrate the ratio of source ingredients (Gelatine, Agar and Starch) will added in different ratio to prepare film and the results of film forming was recorded.

Characterization study of obtained both films were recorded by analyzing the Colour chromaticity, Thickness, TGA (Thermo Gravimetry Analysis), FTIR (Fourier Transform infrared spectroscopy) and Microbial testing.

To increase shelf life of tomato, the produced film was applied in wrapping of Tomatoes to extend their shelf life and to calculate the weight loss % observed in the wrapped tomato for every 3 days to 15 days.

The major advantage of the use of such films obtained from different source are degradable and applied for all food packaging of fruits and vegetables

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CHAPTER 1

1. INTRODUCTION:

The majority of films made for different applications are made of petrochemical polymers and are not biodegradable. Plastic was effectively recycled. 16.7 million tons of plastic packaging totaling 40.9% recycled plastics were collected in the same year.

The negative effects on the environment brought on by the daily disposal of an excessive amount of non-biodegradable garbage are a serious problem. Due to this reality, significant research and development are being done to create new biodegradable film that can be produced using raw materials that are environmentally safe.

Therefore, some other source must be used to replace or lessen this situation. Biodegradable film should be utilized in a variety of applications to address these. With or without oxygen, living organisms like bacteria or fungi can break down biodegradable film without causing any environmental harm.

1.1 BIO BASED POLYMER FOR FOOD PACKING:

It is well known that polymers have a low unit weight and strong shear strength properties, making them promising materials for applications requiring flowable fill. Natural biopolymers can be obtained from renewable agricultural or marine food resources, and they are biocompatible and biodegradable, which contribute to ecological safety. Additionally, they can be prepared in a variety of ways to produce chemically or enzymatically modified derivatives for different end users.

Biochemists, biotechnologists, and physicists have become more interested in the creation and characterization of edible films and coatings due to the wide range of uses for bio-based polymers. Some polymers made from natural resources have previously been developed and put on the market for specific products.

Some of these polymers are edible, and they have historically played, and continue to play, a crucial role in the food, pharmaceutical, and other sectors.

Many of these materials are combined with synthetic polymers or used alone to generate a class of active packaging known as edible films or coatings.

Comparing edible films and coatings to traditional non-edible polymeric packaging, there are many benefits to be had. Due to their biodegradable nature, they can help to reduce environmental pollution even if they are not consumed together with the packaged food. They can also simplify food packaging.

1.2 EDIBLE FILMS AND COATINGS PREPARATION & APPLICATION:

There are two different ways to produce edible coatings. The dry process method, which uses thermoplastic extrusion as an example, relies on the thermoplastic capabilities of polymers when they are plasticized and heated above the temperature at which they transition to glass under low water content conditions. Extruded films have the drawback of being unable to cover uneven surfaces. The second method is known as the wet-process method, which is based on film-forming dispersion and involves first dispersing polymers into a liquid phase before drying them at the conclusion of application. Through dipping, brushing, or spraying, the wet technique enables the direct application of coating in liquid form to food products.

The majority of foods are recognised to be vulnerable to mechanical harm, physiological deterioration, water loss, and rot while in storage. Reduced turgidity brought on by water loss accelerates the loss of nutrients and organoleptic qualities and is a primary factor in degradation. Some items are frequently stored in the refrigerator, whereas others, like fruits, are typically kept at room temperature in markets between harvest and consumption. Product losses will therefore be quite substantial. Spoilage could be reduced with the use of edible coatings and cold storage. Fruits, vegetables, meat, poultry, grains, confectionary items, and diverse foods—all of which can be consumed fresh, frozen, cured, or processed—have all been given edible film treatments.

Controlling mass transfers, providing mechanical protection, and providing sensory protection are the three most crucial functions of an edible film or coating. Controlling mass transfers include keeping foods from drying out, managing the gaseous microenvironments around them, and limiting the movement of components and additives within food systems. By modifying and controlling the internal environment of each meal, edible coatings on fresh foods can serve as an alternative to modified atmosphere storage by minimising quality changes and quantity losses.

By applying edible coatings, one can alter the internal atmosphere and exacerbate disorders brought on by excessive carbon dioxide or low oxygen levels. Even the introduction of oxygen can have a negative impact on food quality due to the oxidation of the meal's fragrance components. For fresh products, edible film with higher water vapour permeability is also preferred, yet exceptionally high water vapour permeability is also not preferred since it may cause fruit to lose too much moisture during storage. To maintain the integrity of the package during distribution, an edible film must have sufficient mechanical strength. The acceptance of finished products is significantly influenced by the sensory qualities of an edible coating or film.

The selection of ingredients is one of the most crucial elements in the creation of edible film. Food-grade coatings and films based on polysaccharides, a class of naturally occurring macromolecules, tend to be very bioactive and are typically made from agricultural waste or shellfish shells. Due to their superior selective permeability to oxygen and carbon dioxide, polysaccharides have mostly been exploited as food coatings.

These inexpensive films are mostly made of cellulose and its derivatives, including ethers and esters, starch, pectin, and gums, all of which have uses in the preservation of food. To prevent the quantity loss of fresh fruit products like tomatoes, cherries, fresh beans, strawberries, mangoes, and bananas, edible coverings based on cellulose have been widely used. Another polysaccharide that is frequently used to prevent post-harvest deterioration in fresh fruits and vegetables is chitosan. Chitin, which is found next to cellulose, is the source of chitosan.

The plant world contains a variety of polysaccharides that can gel in water. Some of them, including pectin in higher plants, agarose and carrageenan in algae, algal and bacterial alginates, and xanthan, have been thoroughly studied. Mucilage's, such as the by-product cactus stems, are often hetero polysaccharides derived from plant stems.

Food, cosmetics, pharmaceutical, and other businesses might use cactus mucilage. Complex polysaccharides are a type of dietary fibre that may absorb a lot of water before dissolving and dispersing to create viscous or gelatinous colloids. The cost-effectiveness of cactus mucilage as a coating is a key consideration.

Edible polysaccharide coatings demonstrated a variety of functions. On cooked pig patties exposed to gamma radiation, green tea leaf extract powder coated with pectin demonstrated improved radical scavenging and lowered lipid oxidation characteristics. In both aqueous and hydroalcoholic media, edible coatings consisting of hydroxypropyl methylcellulose and surfactant combinations were created.

High viscosity, low surface tension, large lipid particle size, and high flocculation rate of the film-forming dispersions all contributed to the films' improved water vapour permeability. Agar can be a useful component that helps to improve the mechanical properties of starch film, as demonstrated by composite edible films made of agar-starch-arabinoxylan polysaccharides mixes. Arabinoxylan may also be used as an addition to improve the effectiveness of agar-based films as moisture barriers.

Fruit of the grape plant was coated with hydroxypropyl methylcellulose edible coatings that included an ethanolic propolis extract. It was discovered that adding propolis improved the nutritious qualities of the coated product and enhanced the colour brilliance of the grapes. Numerous types of polysaccharides, such as cellulose derivatives and various composite coverings, have been the subject of much research. Their use on fruits and vegetables during a thermochemical pre-treatment step produced encouraging results (DIC: Instant Controlled Pressure Drop Process). There is a plenty of material in the literature about this issue.

Although proteins and polysaccharides have the potential to be multifunctional, their hydrophilic nature prevents them from performing the desired roles for edible films. Incorporating hydrophobic chemicals, optimising polymer-polymer interactions, and creating cross-links are a few strategies to enhance the water barrier and mechanical properties of these films.

Edible coatings frequently use lipid molecules such as waxes, which are esters of long-chain monohydric alcohols and fatty acids, and neutral lipids of glycerides, which are esters of glycerol and fatty acids. To give food coatings hydrophobicity, they are added. The numerous work concepts for the production, characterisation, and characteristics of edible coatings are shown in Fig. 1.

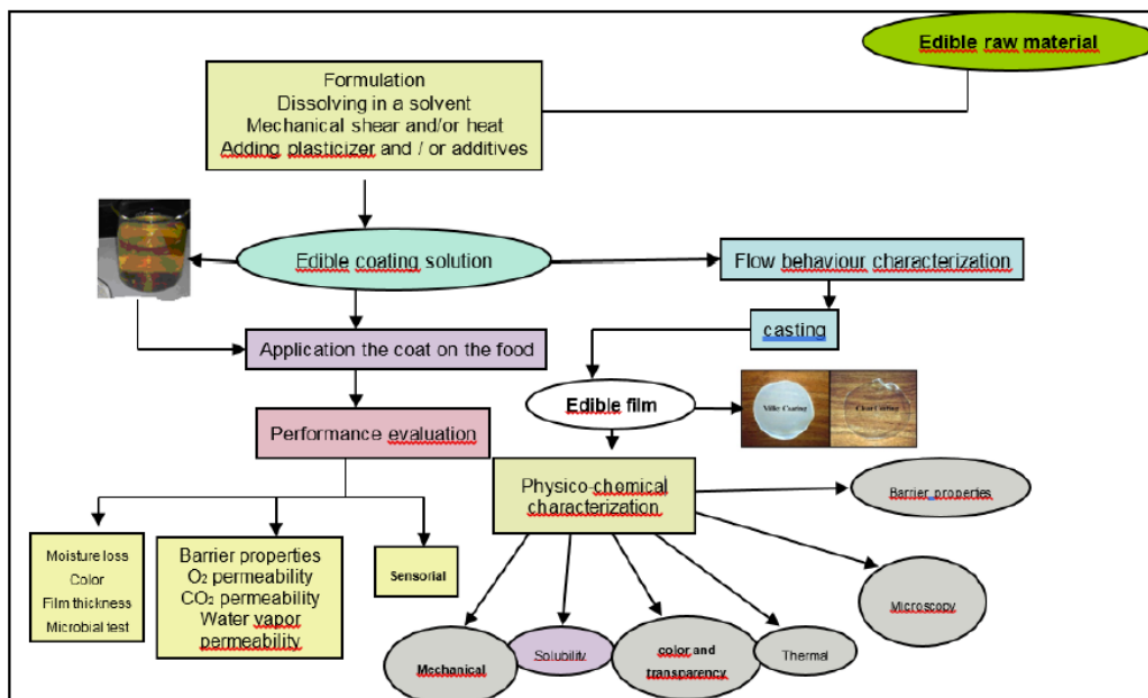


Fig 1.1 Flow chart showing the work concepts of edible films and coatings

1.3 CLASSIFICATION OF EDIBLE FILMS:

Proteins, polysaccharides, lipids, and composites can all be found in edible films and coatings. They are described as thin layers positioned (pre-formed) between food components or created as a coating on a food surface. They serve as a barrier against dangers and increase the food product's shelf life. They can slow the loss of volatile chemicals and moisture, lower respiration rates, and postpone changes in textural qualities.

Additionally, compared to traditional synthetic films, they have a high selective gas permeability ratio CO_2/O_2 and are effective fat and oil barriers. They can also enhance the food's handling qualities and mechanical integrity by serving as transporters for food additives like antioxidants and/or antibacterial agents. For some applications, standalone edible films with high mechanical characteristics could take the place of synthetic packaging films.

1.4 APPLICATION OF FRUITS AND VEGETABLES:

Fruits and vegetables are crucial components of a daily diet and have been increasingly popular among the public in recent years. They are the source of nutrients that are readily destroyed by biotic and abiotic stresses, such as vitamins, vital minerals, antioxidants, bioflavonoids, dietary fibres, and flavouring compounds.

Since fruits and vegetables are highly perishable, there are significant losses during the post-harvest period as a result of microorganisms, insects, respiration, and transpiration. Internal elements include the species, cultivar, and stage of growth whereas external influences include atmospheric composition such as O_2 , CO_2 , ethylene ratios, temperature, and stress factors.

To increase the quality and shelf life of fruits and vegetables, edible coatings are applied to them. Fruits and vegetables can be kept fresher longer by being coated with edible substances. These are safe to consume when consumed with the product and do not alter the food's nutritional value. Fruits and vegetables have a longer shelf life and are more environmentally friendly thanks to edible coatings or films. In order to preserve fresh fruits and vegetables, new edible films and coatings have been created in recent years. These coatings include a variety of edible herbs

as well as anti-microbial substances. edible coatings to stop moisture and firmness loss. They manage respiration rate, growth, and maturation.

In fruits and vegetables like tomatoes, cucumbers, and cherries, among others, edible coatings prevent oxidative browning and slow the growth of microorganisms. By lowering moisture loss, solute migration, gas exchange, and other physiological disturbances, edible coatings or edible films help extend the shelf life of fruits and vegetables. Edible coatings have a strong potential to reduce fruit and vegetable browning, discoloration, off flavours, microbial activity, and shelf life. Baldwin claims that the primary function of edible coating for fruits and vegetables is essentially to strengthen the natural barrier, if it already exists, and to replace it in situations where handling and washing have partially removed or changed it. Moreover, one of the most important things of this edible coating is the fact that they can be eaten together with the fruits and vegetables.

1.5 HISTORY

This is not a novel preservation technique—edible coatings or edible films have been used for millennia in the food industry to preserve food goods. Waxing on fruits and vegetables and cellulose coating on meat casings are two examples. In China, edible coatings have been in use since the 12th century. Fruit waxing was created and used for the first time on fruits and vegetables in a commercial setting in 1922. A barrier for chemical, physical, and biological changes is formed by edible films and coatings.

When buying fruits and vegetables, consumers evaluate the quality and freshness of the food based on how it looks. In the fresh cut fruit industry, maintaining and controlling fresh quality, growth of spoilage, and pathogenic microorganisms are the most prevalent and difficult issues. Edible coating is the answer to this issue. Fresh fruits and vegetables benefit from an additional layer of protection from edible coating, which can have the same impact as modified environment storage in terms of altering internal gas composition. Many edible coatings have recently been effectively used to preserve fruits and vegetables, including oranges, apples, grapefruits, cherries, cucumbers, strawberries, tomatoes, and capsicums. The control of what goes into the edible coating of fruits and vegetables determines whether it is successful or not.

1.6 DEFINITION:

The term "edible coating" refers to a thin layer of material that can be eaten while also protecting food from oxygen, external pathogens, moisture, and solute movement. In edible coating, a semi-permeable barrier is offered with the goal of extending shelf life by lowering gas exchange, oxidative reaction rates, respiration, moisture and solute migration, and physiological diseases on fresh cut fruits.

According to Pavlath and Orts, numerous fruits and vegetables have been coated or wrapped in a variety of materials to increase their shelf life. Whether removed or not, these coatings are deemed edible and can be consumed with food. Fruits and vegetables have a dazzling appearance thanks to edible coatings or films. The average edible covering is less than 0.3 mm thick. Edible coating's primary trait is its ability to extend the shelf life of fresh or processed fruits and vegetables and to protect them from postharvest losses and environmental deterioration.

Fresh fruits and vegetables have an edible outer membrane that serves as protection. The edible coatings are utilised as a nutraceutical and as a carrier of antioxidants and texture enhancers. The edible coating should be stable and usually regarded as safe even in conditions of high relative humidity. The majority of edible coatings and films are flavourless, colourless, and odourless, and they ought to have high mechanical properties. The gas barrier and moisture barrier qualities of edible coatings are good.

1.7 PROPERTIES:

The molecular makeup, molecular size, and chemical composition of edible coatings determine their physical and chemical characteristics. The characteristics are as follows: Edible coatings offer good moisture, oxygen, carbon dioxide, and ethylene barrier qualities.

It enhances aesthetic and mechanical handling to preserve fruit and vegetable structure and colour.

Active ingredients included in edible coatings, such as vitamins and antioxidants, improve the nutritional content of fruits and vegetables without degrading their quality.

These coatings extend the shelf life of fruits and vegetables by providing a protective covering.

1.8 FRUITS AND VEGETABLES SUITABLE FOR COATINGS

Fresh fruits and vegetables are extremely perishable, and during harvest, processing, transit, and storage, about 50% of fresh food degrades. The use of edible coatings is crucial in handling this circumstance. Whole and freshly cut fruits and vegetables receive edible coverings. Those covered fruits and vegetables are

Fruits : Fresh-cut Apple, Fresh-cut Peach, Fresh-cut Pear, and other edible coated fruits include Orange, Apple, Grapefruit, Cherry, Papaya, Lemon, Strawberry, Mango, and Peach.

Vegetables: fresh-cut potatoes, fresh-cut cabbage, fresh-cut tomato slices, fresh-cut onion, lettuce, cantaloupe, cucumber, capsicum, and tomato.

1.9 CLASSIFICATION OF EDIBLE FILM AND COATINGS

To increase the function of edible coatings, hydrophilic groups such as polysaccharides or protein or hydrophobic groups such as waxes or lipids are sometimes included. These groups can also be combined. Edible coatings are not organic or chemically created. Fruits and vegetables are typically preserved and given a good appearance using this method. The key benefits of edible coating over other synthetic coatings are that it is edible, non-toxic, and affordable. Polysaccharides, proteins, and lipids make up the majority of edible coating ingredients. The edible coatings are primarily categorised into three groups. Those are

Hydrocolloids : Polysaccharides, Proteins and alginate

Lipids : Fatty acids, Acryl glycerides and waxes

Composites : Protein /Protein, Polysaccharides /protein, Lipin /Polysaccharides

By adding surfactants and plasticizers, edible coating materials are made from a variety of natural ingredients, including polysaccharides, protein, and lipids. The mechanical, barrier, and colour features of edible coatings, which regulate gas movement and moisture loss of fruits and vegetables, are largely responsible for their function and performance.

1.10 HYDROCOLLOIDS :

Hydrocolloids are hydrophilic polymers that can be derived from animals, plants, microorganisms, or synthetic materials. They include a hydroxyl group and can be polyelectrolytes such as gum Arabic, alginate, carrageenan, pectin, and carboxymethyl cellulose. It has a wide range of applications as a coating forming solution to coat and regulate the colour, texture, flavour, and shelf life of fruits and vegetables.

All hydrocolloids are often partially or totally dissolved in water, and their main application is to thicken gelling agents by raising the aqueous phase's viscosity. Because of this stabilising function, they serve as an emulsifier. There are two categories for hydrocolloids. Those are

- a. Polysaccharide – based
- b. Protein based

1.10.1. POLYSACCHARIDE – BASED FILMS

The most popular polysaccharides used to coat fruits and vegetables for consumption include chitosan, starch, alginate, cellulose, pullulan, carrageenan, and gelatin. Food coatings made of polysaccharides have subpar moisture barrier qualities. It dissolves in water. However, it has a mediocorely poor oxygen permeability. Fresh, minimally processed fruits and vegetables are coated with edible coatings made primarily of polysaccharides in order to slow down their respiration. increased mechanical handling characteristics and carrying capacity of the additives. A variety of edible coatings benefit from the crispiness, hardness, compactness, thickening quality, adhesiveness, and viscosity provided by polysaccharides. Fruits' shelf lives are extended by polysaccharides because of their strong gas barrier qualities, which are made up of polymer chains.

In order to lengthen the shelf life of fruits and vegetables without creating anaerobic conditions, polysaccharides are composed of polymer chains that have strong gas barrier qualities.

Derivates of Cellulose: Cellulose can be easily found in nature. Long chains of the polymer anhydro-glucose are used to make it. After being treated with the

proper chemical reagent and reacting with an alkali, cellulose is transformed into a replacement cellulose chain of anhydro-glucose monomers. This procedure was carried out in a controlled environment. Below are the replacement reagents

- a. Methyl cellulose – Formed by chloromethane
- b. Hydroxypropyl cellulose (HPC) – substitute reagent is propylene oxide
- c. Hydroxypropyl Methyl cellulose (HPMC) – mixed substitution of chloromethane & propyl oxide.
- d. Methyl ethyl cellulose (MEC) – mixed substitution of chloromethane & chloroethane.
- e. Carboxy Methyl cellulose (CMC)

Methyl cellulose, HPMC, HPC, CMC films or coatings film forming characteristics

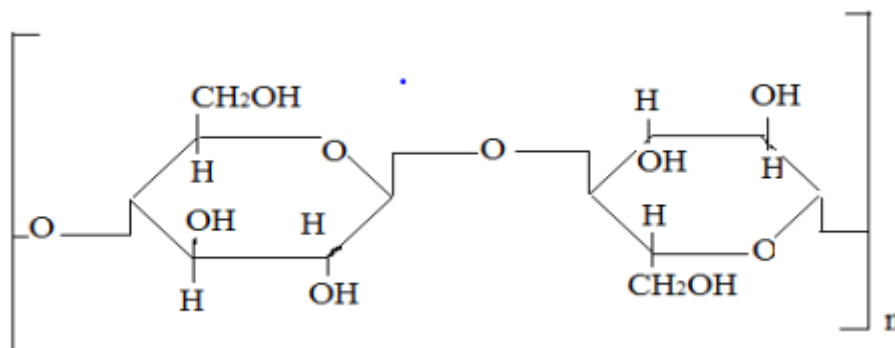


Fig.1.2 Structure of cellulose

The building blocks for cellulose derivatives are polymer chains comprised of two anhydro- glucose or -glucopyranose repeating units that are pulled together by a 1, 4-glucosidic bond. The number n in this chemical structure denotes the number of polymerized glucose monomers. The average number of -OH groups that are substituted for one anhydroglucose unit is known as the degree of substitution.

Typically, modified cellulose derivative edible coatings and films provide transparent solutions that are colourless, odourless, and flavourless. It has excellent fat and oil resistance, is soluble in water, and is only moderately permeable to

moisture and gas transmission. The molecular weight of cellulose determines the mechanical barrier property of edible coatings and films, the higher the molecular weight of cellulose, the better the barrier qualities.

Methyl cellulose is the least hydrophilic of those materials and has high moisture barrier properties. These modified cellulose derivatives are hygroscopic in nature and come in granule and powder form. However, the gas and water barrier qualities of coatings and films made of cellulose derivatives are subpar. Derivatives of polysaccharides are hydrophilic by nature, which accounts for their subpar mechanical qualities.

1.10.2 STARCH DERIVATIVES:

The most prevalent polysaccharide is starch. Cereal grains, which include wheat, maize, rice, and others, are members of the poaceae family and are a source of starches. Legumes, along with potatoes and other tubers, are excellent sources of starch. The units of anhydro-glucose make up starches. One of the components of starch is the water-soluble polysaccharide amylose, while the other is the water-insoluble molecule amylo-pectin.

However, the amylopectin is a branched chain of glucose, whereas the amylose are a linear or unbranched chain of D-glucose (1-4 linkage) (1-6 linkage). Amylose is a useful source of starch for use in coating and film. High amylose maize starch or potato starch coatings and films have high levels of stability for long-term preservation.

Glycosidic linkage holds these chains together. Amylase aids in the breakdown of starch derivatives, producing dextrin as a byproduct. Corn starch, for instance, is a great source of starch amylose for coating and film production. The physical features of corn starch-based films and coatings have been compared to those of plastic films and coatings. They are tasteless, odourless, colourless, and non-toxic. It is physiologically absorbing and somewhat permeable to carbon dioxide and oxygen.

1.10.3 AGAR:

Red seaweed belonging to the Rhodophyceae family is used to make agar gum. A galactose polymer, that is. A mixture of agro pectin and agrose is found in agar, which is hydrophilic and colloidal in nature. It is frequently used to add hardness to microbiological media. For temperatures above the initial gelation temperature, agar forms a strong gel with a melting point. Agar gels are placed in a cold environment and melt in a hot environment. because of its extraction and ability to form a firm gel at low concentrations. The food business uses agar frequently.

1.11 PROTEIN BASED EDIBLE COATING:

Animals and vegetation both provide protein for edible coatings. Egg albumen, collagen, and other animal-based proteins are used as edible coating materials. Plant-based proteins include milk protein casein, whey protein, zein (from maize), gluten (from wheat), and soy protein. An edible coating made of protein has great barrier qualities for fragrance, oil, and oxygen and provides strength, but it is ineffective against moisture. The closely packed hydrogen linked structure of oxygen is what gives it such a good barrier characteristic. At low relative humidity, protein-based edible coating provides high oxygen barrier properties. Due to their hydrophilic nature, these coatings are poor water vapour barriers, although they do have strong organoleptic and mechanical qualities.

1.12 PLASTICIZERS:

Plasticizers are added to an edible coating solution to improve the coating's mechanical properties. These have a low molecular weight and are coated with protein to modify their structural capabilities. Additionally, water is a powerful and natural plasticizer. The three most often added plasticizers to coatings are glycerol, fatty acids, and sorbitol. Sucrose, polyethylene glycol, monoglycerides, and propylene glycol

CHAPTER 2

2. LITERATURE REVIEW:

By utilizing agro-based polymers and other biomaterials found in nature, the environmental and sustainability issues raised by the usage of synthetic polymer materials can be significantly reduced.

Starch, chitin, chitosan, agar powder, gelatin, and pectin are just a few examples of the plant and animal-derived polymers that are thought to offer viable feedstock for novel materials. These polymers have a high density of surface-active functional groups, such as hydroxyl, amino, and carboxy groups, which makes it easier for the material to generate intermolecular hydrogen bonds.

In-depth research is being done to adapt these renewable resources to the production of high-performance biomaterial for various commercial applications. When synthetic film is released into the environment, it will cause severe environmental contamination and harm to species.

2.1 MATERIALS USED FOR FILM PRODUCTION

When French scientist Francois Mothers obtained a patent for gelatine capsules to mask the bitter taste of pharmaceuticals, gelatine (Gelatus) was first used as a pharmaceutical product. The first industrial gelatine for glue was introduced in France in 1818.

Agar is a polymer of agarobiose, a disaccharide comprised of galactose and 3,7-anhydrogalactose, which is produced by the bacterium *Gelidiella acerosa*.

In British English, glycerine is another name for glycerol. It is a thick, colorless syrup that was created in 1838 by French scientist Michel Eugene Chevreul from the combination of the words glycerol and chemical. Its sweet flavor gave it this name. In addition, it is frequently employed as a humectant in

pharmaceutical formulations and as a sweetener in culinary products. Glycerol is hygroscopic in nature and miscible with water due to its three hydroxyl groups.

A polymeric carbohydrate called starch (amylum) is made up of many glucose units connected by glycosidic linkages. Most green plants synthesize this polysaccharide as a form of energy storage.

2.2 PLASTICIZER:

Film formation and plasticization. It describes a shift in a polymer's thermal and mechanical properties that involves a reduction in the stiffness at room temperature, a decrease in the temperature at which significant deformations can be caused with manageable forces, and a rise in the elongation to break at room temperature. To achieve these effects, a given polymer can be combined with another polymer or a low-molecular-weight chemical, and a commoner that increases chain flexibility and decreases crystallinity can be added to the original polymer.

The term "plasticizer" typically refers to the intended characteristics of a particular polymer plasticizer system. It is described as a substance for films that provides stress resistance, flexibility, and improved film workability. It is a substance that, for plastics, provides a desired level of flexibility across a broad temperature range and lowers the brittle points.

However, it doesn't appear possible to describe plasticizer behavior in terms of certain fundamental characteristics. This is so because the polymer to which the plasticizer is applied has a significant impact on how it behaves. The concentration of a plasticizer has a significant impact on how effective it is. A study discovered that the relationship between the concentration and molecular structure of a plasticizer in a polymeric material and the reduction of T_g in films is direct. Despite opposition from certain researchers, it has since been discovered that molar concentrations can be used to compare the efficacy of plasticizers.

There is often a molecular weight range for each plasticizer, such as the sebacates, adipates, or phthalates, with a particular polymer like polyvinyl chloride when plasticizer effectiveness is at its best. The polarity of plasticizer molecules may also have an impact on how well they work. To obtain good compatibility, polar plasticizer must be used with polymers that contain polar groups. As the distance between polar groups throughout the polymer chain increases reduced, there is a greater likelihood that plasticizer polarity will triumph over the interactions between polymer molecules.

When the plasticizer's molecular forces are greater than the interactions between its polymer molecules, however, no plasticization takes place. Additionally, it was accepted that polar groups on aliphatic molecules rather than aromatic ones promote better plasticization. The more mobile aliphatic molecules are to blame for this (Dyson, 2003).

Numerous research has documented how sorbitol and glycerol plasticize different sources of starch for creating biodegradable or edible films (Bakry et al., 2017; Nor et al., 2017; Soo & Sarbo, 2018). To successfully plasticize gelatin film, glycerol or another suitable plasticizer must be present in amounts of at least 20%. (Pushpadass, Marx, & Hanna, 2008). Tensile strength, young's modulus, and glass transition temperature (T_g) decrease with an increase in plasticizer content, but elongation and gas permeability rise.

A gelatin film with 25% glycerol is said to have the highest tensile strength and best elasticity module (Pushpadass et al., 2008). However, Hanani et al. (2014a, 2014b) discovered that when used as a plasticizer only, glycerol molecules can migrate from film matrix. Both Zhong and Xia (2008) and Jongjareonrak, Benjakul, Visessanguan, and Tanaka (2008) noted similar patterns with gelatin films plasticized with glycerol alone.

In the instance of bovine gelatin films, Sorbal, Monterrey-Q, and Habitante (2002) found that the gelatin film is easily fractured when sorbitol alone is utilized as the plasticizer. Bergo et al. (2013) noted that employing glycerol and sorbitol together rather than the two substances separately resulted in the production of films with stable film characteristics.

Mali et al. (2005) claim that strong plasticizer-plasticizer interactions result from the usage of mixed plasticizers in film matrix, which can enhance specific film qualities. Al-Hassan and Norziah (2012) also discussed the importance of mixing two plasticizers, particularly polyols, at various concentrations to compare how well they produce gelatin-based films.

Plasticizers for biopolymer-based films might be divided into two categories: those that were water soluble and those that were not (Siepmann, Peeratakul & Bodmeier 1998). The amount and types of plasticizers had a significant impact on how well the polymer dispersions in the aqueous solution formed films (Johnson, Hathaway, Leung, & Franz 1991).

When added to a polymer, hydrophilic plasticizers dissolved in the aqueous solution; however, if supplied in large quantities, this could promote water diffusion in the polymer (Hanani et al., 2014a, 2014b). Hydrophobic plasticizers, on the other hand, could seal off the film's micropores, reducing the amount of water absorption. Water insoluble plasticizers, however, have the potential to produce phase separation, which results in a loss of flexibility, or the development of discontinuity zones during film drying.

Due to environmental considerations, natural biopolymers, such as gelatin, agar, starch, and glycerin, among others, can generate edible and/or biodegradable films and serve as an alternative material to those plastic films made from synthetic polymers. Numerous studies are being conducted to enhance certain biopolymer-based film features, such as their mechanical, gas (O₂, CO₂), and water vapor barrier capabilities.

2.3 GELATIN

Collagen that has been extracted from animal skin and bones is thermally denatured with a very diluted acid to produce gelatin. Nearly one in three of the residues in the gelatin structure are glycine, which is also found in proline and 4-hydroxyproline. A typical gelatin structure is (-Ala-Gly-Pro-Arg-Gly-Glu-4Hp-Gly-Pro-). Dehydration causes conformational changes that can be utilized to create surface films. These movies perform best when they include more triple-helix content. These films are made of hygroscopic materials, making them particularly sensitive to environmental factors like water vapor. In these films, the water molecules have potent plasticizing properties.

Additionally, plasticizers like polyols (Sorbitol, glycerol, xylitol, etc.) are used to change the functional characteristics of these films. The plasticizer modifies the strength of the interactions between adjacent macromolecules by extending the distance between them and thereby reducing the internal forces at a microscopic level, which increases the films' flexibility at a macroscopic level. Additionally, due to the mobility of the polymeric chains, the plasticizer shifts the glass transition (T_g) of the films toward lower temperatures.

The capacity of a polar molecule, dipole (permanent and/or induced), or spatial charges to follow the rapid fluctuations of an applied electric field is referred to as a dielectric's (insulator's) polarizability. Due to the macromolecules' largest size, it is reasonable to anticipate that their polarizability will be minimal. Due to the hydrophilic nature of these materials and the presence of Materials Science Forum, the water molecules may be the primary source of polarizability in edible films.

2.4 AGAR:

Agar is a polysaccharide that is obtained from marine red algae species that are members of the Rhodophyceae family. The two primary commercial sources of this polymer are *Geledium* sp. and *Gracilaria* sp. In the seaweeds' cellular walls, agar creates a supporting structure.

The non-gelling fraction of agaropectin and agarose make up the chemical structure of agar (gelling fraction). The former is somewhat branched and sulfated, whereas the latter is a linear polysaccharide made up of repeated units of D-galactose and 3,6-anhydro-L-galactose, connected by alternating $-(1 \rightarrow 3)$ and $-(1 \rightarrow 4)$ glycosidic linkages (Fig. 1.1). When agar is manufactured commercially, agaropectin is typically eliminated, yielding an agar powder with a stronger gel.

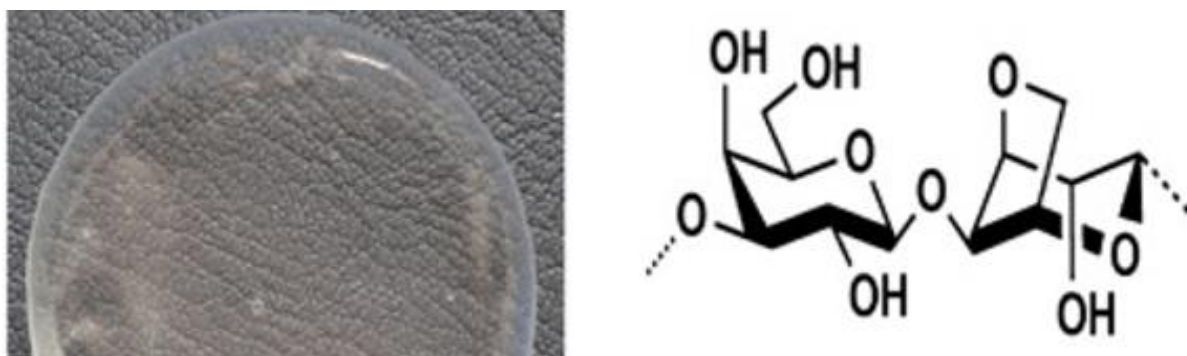


Fig. 2.1 Agarose film and its chemical structure

Agar powder comes in a variety of forms with different sources for the algae used in its manufacturing as well as different qualities and uses. The standard industrial procedure for extracting agar gum calls for boiling dry algae to release the agar gum that is present in the cell wall, dissolving it in water, sifting the liquid extract, and then using freezing and thawing methods to separate the gum from the water. Agar's capacity to gel could also be enhanced by an alkali treatment.

L-galactose-6-sulphate is modified throughout this process, and 3,6-anhydro-L-galactose is what gives gel its strength. Alkali treatment, however, can have a

deleterious impact on the films that were produced. The properties of films formed from natural and alkali-modified agar gums were compared by Sousa & Souza.

They claimed that, when extracted on an industrial scale, native agar was less expensive and safer. Additionally, it offered a higher viscosity than the alkali-modified ones. The films created from native agar gum also shown greater elasticity and superior water vapor barrier qualities while looking more vibrant.

Agar's capacity to continuously produce films is predicated on its propensity to gel (Fig2). When agar powder is dissolved in hot water, a viscous liquid is created. The development of thermoreversible gel may occur if the temperature is lowered below the gelling range (90-103 C).

Only the creation of hydrogen bonds between agarose molecules causes the physical gelation of agar. A network of agarose double helices is created in the agar gel and is supported by water molecules. They unite through the hydroxyl groups on their outside. Agar gels have the unique ability to gel, which allows them to retain a significant amount of water within their network.

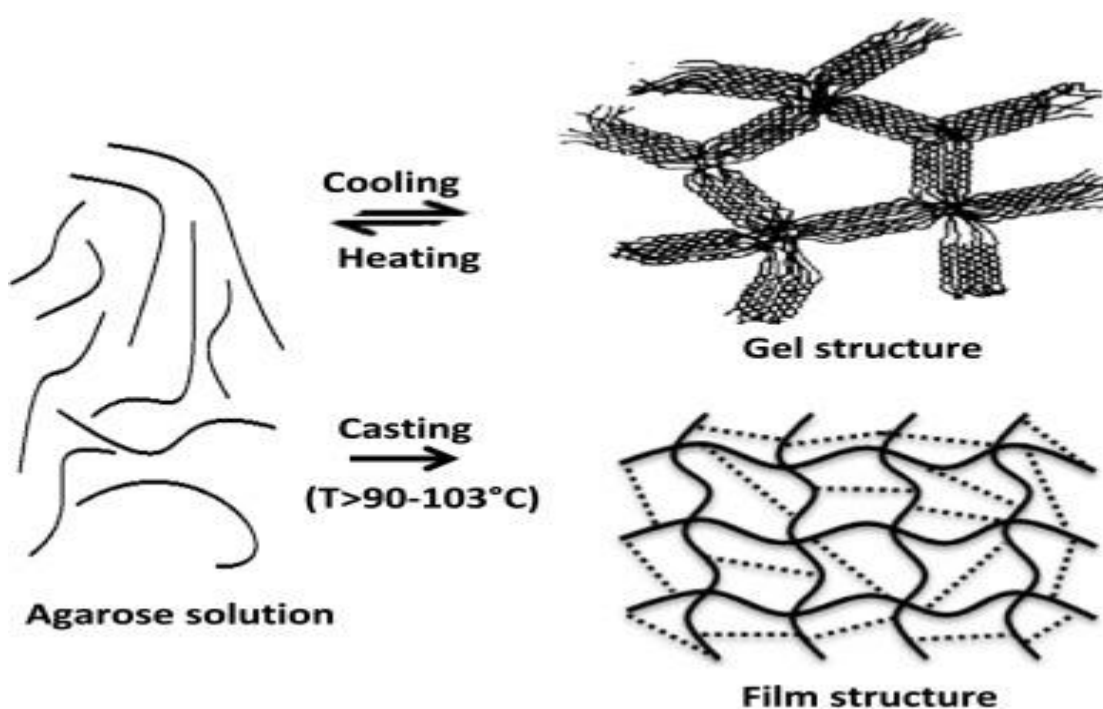


Fig 2.2 Agar Gel Structure and Film Structure

2.5 STARCH:

The most common plant polysaccharide, starch, has been extensively used in the creation of edible coating films due to its availability, affordability, and superior film-forming properties. Films made of starch have excellent optical, organoleptic, and gas barrier qualities but low mechanical properties.

The mechanical and tensile properties of the films have been improved by the inclusion of co-biopolymers or other secondary additives, which is one method used to get around these restrictions. Numerous variables, such as the types of starches used, the temperature and length of time during film creation, plasticizers, cobopolymers, and storage conditions, can affect the properties of starch-based films.

A polymeric carbohydrate called starch is made up of connected 1, 4-linked d-glucose units. The two main types of starch polymers, amylose and amylopectin, which together make up 98–99% of the dry mass of starch, can be further separated into semi-crystalline and amorphous layers.

Amylose (25–30%), which mostly contributes to the amorphous phase of the starch granule, is largely a linear, 1,4-linked polymer with sparse 1,6-branches (1%). Amylopectin, in contrast, has a highly branching structure with 1,4-linked spines and 1,6 branch points spaced out every 20–30 glucose units. The organisation of starch granules' peripheral crystals is mostly facilitated by amylopectin.

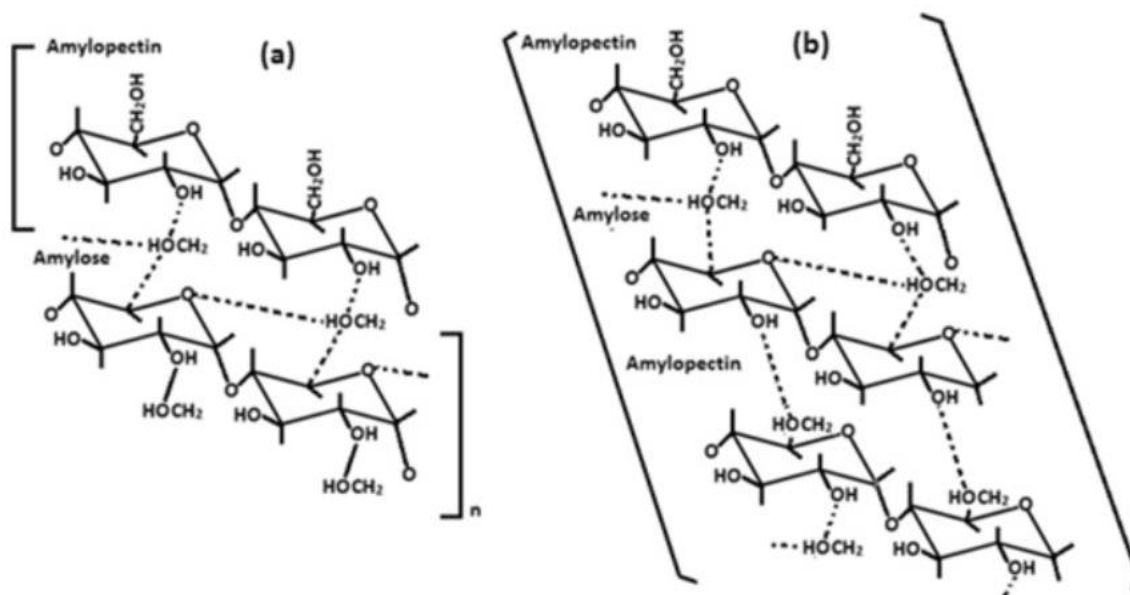


Fig. 2.3 (a) Hydrogen bonding between amylose and amylopectin molecules. (b) Retrogradation mechanism of starch. AY – amylose, AP – amylopectin, dotted lines – hydrogen bonding.

The thermal characteristics and retrogradation profile of starches are influenced by the unit chain length distribution pattern in the internal structure of amylose and amylopectin. In this sense, the characteristics of the starch-based film may be influenced by the composition of these components. The mechanical strength, elongation, and gas barrier properties of films with higher amylose concentrations are often superior.

2.6 GLYCEROL:

Glycerin is another name for the fundamental polymer glycerol. Glycerol is a thick, colorless liquid that has no odor and a sweet taste. Triglycerides, or triglycerides, are lipids with a glycerol backbone that are present in certain lipids. Triglycerides, a type of lipid that includes glycerin, can come from both plant and animal sources. It has three hydroxyl groups, which makes it water soluble. Glycerol has the chemical formula $C_3H_8O_3$. Its compound has several hydroxyl groups. Three hydroxyl groups, or -OH groups attached to the carbon atoms, can be found

in glycerol's chemical structure. It has 8 Hydrogen atoms, 3 Carbon atoms, and 3 Oxygen atoms.

The structural and extended chemical formulas for the formula are provided. Glycerin, commonly known as glycerol, is a viscous, harmless, colorless triol molecule.

A triglyceride known as glycerol is found in many plants, including soybean, palm, and animals. It can also be produced through industrial processes such as saponification.

It is an organic poly compound with the IUPAC name 1,2,3-Propanetriol because it contains a variety of hydroxyl groups and carbon atoms. It is a trihydroxy sugar alcohol because it contains three carbon atoms and three hydroxyl groups.

Glycerol is soluble in water as a result of the hydroxyl (-OH) groups attached to the carbon atoms. These hydroxyl groups, which ensure that glycerol quickly absorbs or retains water, are also a result of the substance's high degree of hygroscopicity.

Glycerin has a three-hydroxyl group and three-carbon atom structure. Each hydroxyl group in this structure is covalently linked to a carbon atom. Below is a diagram illustrating glycerin's structure.

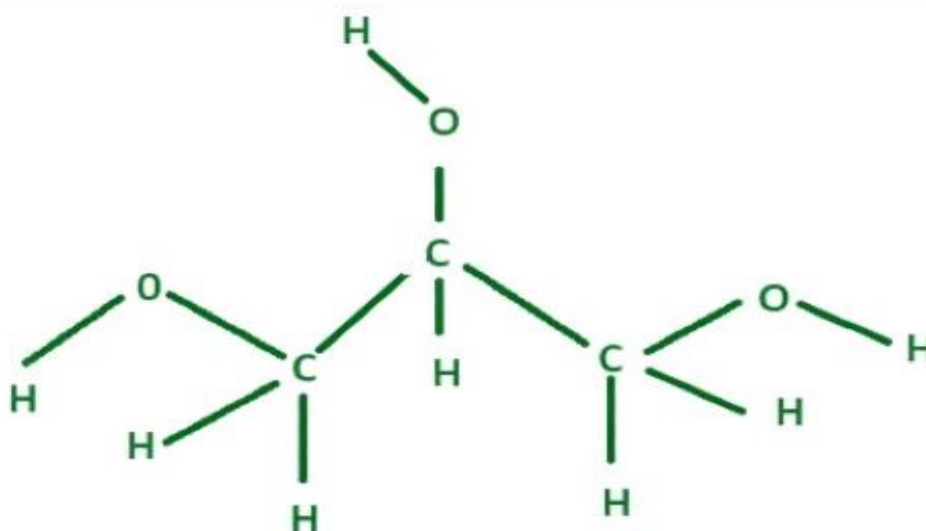


Fig. 2.4 Structure of Glycerin

Glycerin or glycerol occurs as an odorless, colorless, hygroscopic liquid under some typical parameters for temperature and pressure (STP). This glycerol compound can mix with water to generate a miscible combination.

Glycerin's hygroscopic properties, in particular can cause mild irritation to the skin, eyes, nose, lungs, and breathing passages. Pure glycerol can dry out skin and other internal bodily organs when it comes into close touch with these moist tissues.

The same trait that makes glycerol a good humectant also desiccates internal tissues is the molecule's ability to bind to water. On the other side, glycerol can also stop a lotion, cream, or gel from fast drying up if a cosmetic product with a high-water content is applied to the skin, particularly in arid regions.

CHAPTER 3

3. AIM AND SCOPE:

Based on Literature review taking account of all advantages of edible film were resulted in prevention of fruits and vegetable. In this experiment, the film was being prepared in the experiment by using as 2 trails. First trail is based on Film from only Gelatin source, where 100 % of source ingredients Gelatin for process, where Glycerol used as a plasticizer.

Second trails is based on Film prepared by Composite of source material like Gelatin, Agar, and Starch, where all three ingredients area added in different ratio, Where same Glycerol used as plasticizer for this trail also. Trail of composite sample was undergone for different ratio and obtained film will undergo for several testing and application in the vegetable.

Fruits and vegetables are highly perishable as an application part and during post- harvest period, there are significant losses because to microorganism, insects, respiration, and transpiration. The internal elements are species, cultivar, and its growth stage, while the external factors are the atmospheric composition, including the ratios of O₂, CO₂, Ethylene, Temperature, and stress factor.

The flesh of fruits and vegetables can also be contaminated by the skin, causing biochemical deterioration such as browning, off flavors and textual breakdown. This lowers the quality of the produce and increase the risk to customers due to the presence of pathogenic microorganism

To overcome this shelf life extension and prevention of Fruits and vegetables. This experiment was done by testing the obtained film with Vegetable Tomato. The Objectives of this Research is to

- i. To Obtain Film by using Composite of different source of ingredients.
- ii. To Compare the obtained composite film with the Film obtained from Single source Gelatine
- iii. To do experiment on application of obtained both film in increase the shelf life of vegetable -Tomato by wrapping

CHAPTER 4

4. MATERIALS AND METHOD

4.1 MATERIALS

Gelatine was purchased from Acme chemicals Mumbai with Purity 99 % of grade standard 120 Bloom. Agar was purchased from Anton scientific glass works of Lab grade with purity 99.50%. Starch powder procured from STDN Food & Beverages private limited of gluten free grade with purity 99.3%. Glycerol used as a plasticizer was purchased from Maya Chemtech India of Food grade with 99.75% purity.

4.2. FILM PREPRARATION PROCESS

Film was prepared by using two different sources of raw material to study the characterization of film. Process Flow of preparation of film was given below.

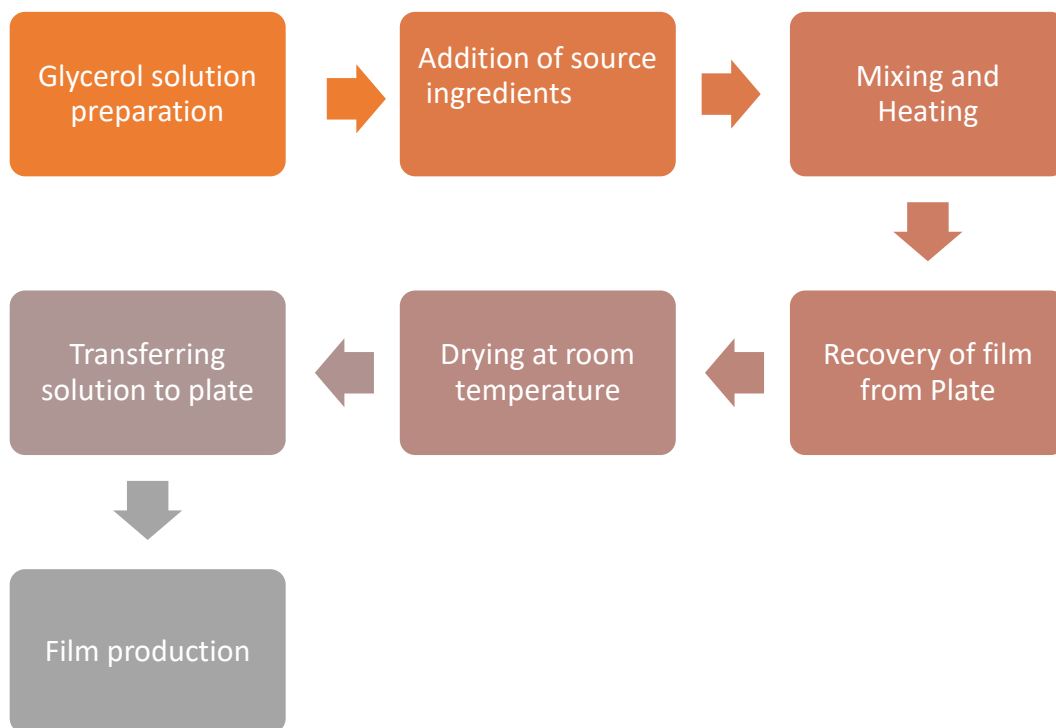


Fig. 4.1 Flow chart for Film preparation process

4.2.1 FILM FROM GELATIN

The films were prepared using casting techniques. Firstly, Glycerol solution was prepared by adding 4 ml of Glycerol in 1000ml of distilled water under manual stirring for 5 min. Secondly, 7 Grams of gelatine was mixed with 360 ml of glycerol solution by manual stirring for 3 min. All mixtures were stirred at 95°C for 15 min to obtain homogenous solution. Thirdly the solution was poured into plate and leave it to dry for 96 Hours at room temperature.

4.2.2 FILM FROM COMPOSITE OF GELATIN, AGAR & STARCH

The films were prepared using casting techniques. Firstly, Glycerol solution was prepared by adding 4 ml of Glycerol in 1000ml of distilled water under manual stirring for 5 min. Secondly, 2.33 Grams of gelatin, Agar & Starch of each was mixed with 360 ml of glycerol solution by manual stirring for 3 min.

All mixtures were stirred at 95°C for 15 min to obtain homogenous solution. Thirdly the solution was poured in a plate and leave it to dry for 96 Hours at room temperature.

As a result of this experiment, two films were produced from two difference source. Final obtained film will be named as below.

1. Film from Gelatine

2. Film from composite of Gelatine, Agar & starch

4.2.3 RATIO OF INGREDIENTS USED IN FILM PREPARATION:

Table 4.1: Ratio of Ingredients

Process	Types of Films	Source	% of Ingredients	Grams used for Production	Plasticizer
Method 1	Film from Gelatine	Gelatine	100	7	Glycerol solution
Method 2	Film from Gelatine, Agar & Starch	Gelatine	33.33	2.33	Glycerol solution
		Agar	33.33	2.33	
		Starch	33.33	2.33	

4.2.4 TRAILS ON FILM PREPARATION AND ITS RESULTS

However, in this experiment we had made different trail in film producing process. The concentration / ratio used as different for film production. It has been experimented of 5 trails by differing the ratio of source ingredients like Gelatin, Agar and starch. Out of this five trails, Only Trail 1 and trail 2 provide positive results in obtaining film. Remaining three trails have resulted in negative. Below table will illustrate detail of trail and ratio of each trail.

Table 4.2: Trail results on film generation from different sources and in different ratio

TRIAL OF FILM GENERATION WITH DIFFERENET RATIO OF INGREDIENTS				
Process	Types of Films	Source	% Ratio of Ingredients	Results
Trial 1	Film From Gelatine	Only Gelatine	100	Success
Trial 2	Film From Gelatine, Agar & Starch	Gelatine	33.33	Success
		Agar	33.33	
		Starch	33.33	
Trial 3	Film From Gelatine, Agar & Starch	Gelatine	50	Failure
		Agar	25	
		Starch	25	
Trial 4	Film From Gelatine, Agar & Starch	Gelatine	50	Failure
		Agar	25	
		Starch	25	
Trial 5	Film From Gelatine, Agar & Starch	Gelatine	50	Failure

CHAPTER 5

5.1 RESULTS AND DISCUSSION

5.1. CHARACTERISATION OF FILM:

The obtained two films has been characterized by using the below parameters.

- a. Color
- b. Thickness
- c. Compositional analysis by thermogravimetry (TGA)
- d. Identification by infrared spectrophotometry
- e. Microbial Testing

5.2 TESTING METHODS OF FILM

Table 5.1 Test methods applied for quality evaluation

TESTING METHOD APPLIED FOR FILMS		
S. No	TEST PARAMETERS	TEST METHODS
1	Chromaticity value (Color)	ASTM D2244:2016
2	Thickness	ASTM D6988 CI 8.4 : 2013
3	Compositional Analysis by Thermogravimetry (TGA)	ASTM E1131:2014
4	Identification by infrared spectrophotometry	ASTM E1152: 2021
5	Microbial Testing	APHA 5 th Edn 2015- Chap3
a	Staphylococcus aureus	
b	Salmonella	
c	Escherichia coli	
d	Total plate count	
e	Yeast and mould	

5.3 TESTING AND RESULTS:

5.3.1 CHROMATICITY VALUE COLOR

Color (Chromaticity indexes) is an important factor that's shape ultimate decision made by consumers in using edible & biodegradable film. The films appeared to have different colors when analyzing them by the b^* , a^* and L^* factors. It was observed that compositing different sources of material made increase in L^* , whereas it has been resulted decrease in a^* and B^* Factor. If lower values of L^* and a^* detected, thereby indicating that the films appear darker. Significant rise in b^* denotes that film are inclined towards yellowness, whereas in this case there is decrease B^* denotes that the film is not inclined towards yellowness.

Table 5.2 Chromaticity data of film

Film sample	Chromaticity indexes			Condition
	L^*	a^*	b^*	
Film from Gelatine	85.6	- 0.47	3.25	D65/2°C
Film from composite of Gelatine, Agar, and Starch	88.01	- 0.16	-1.81	D65/2°C

5.3.1.1 Color chromaticity indexes of films a^* , b^* & L^* factors :

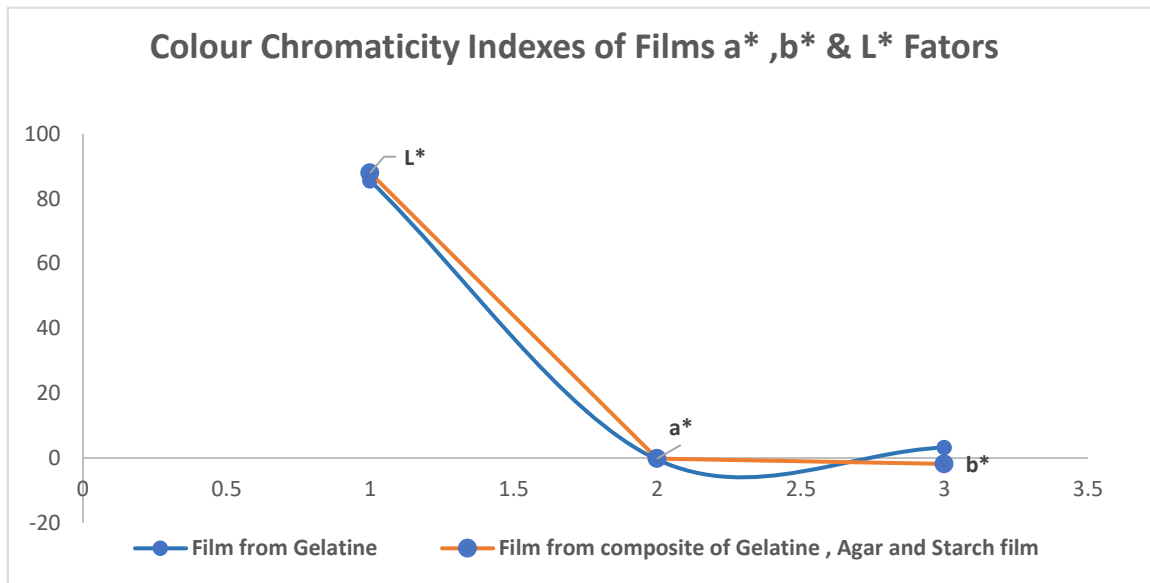


Fig. 5.1 Colour chromaticity indexes of films a^* , b^* and L^* factor

5.3.1.2 Chromaticity indexes of film by L^* factor:

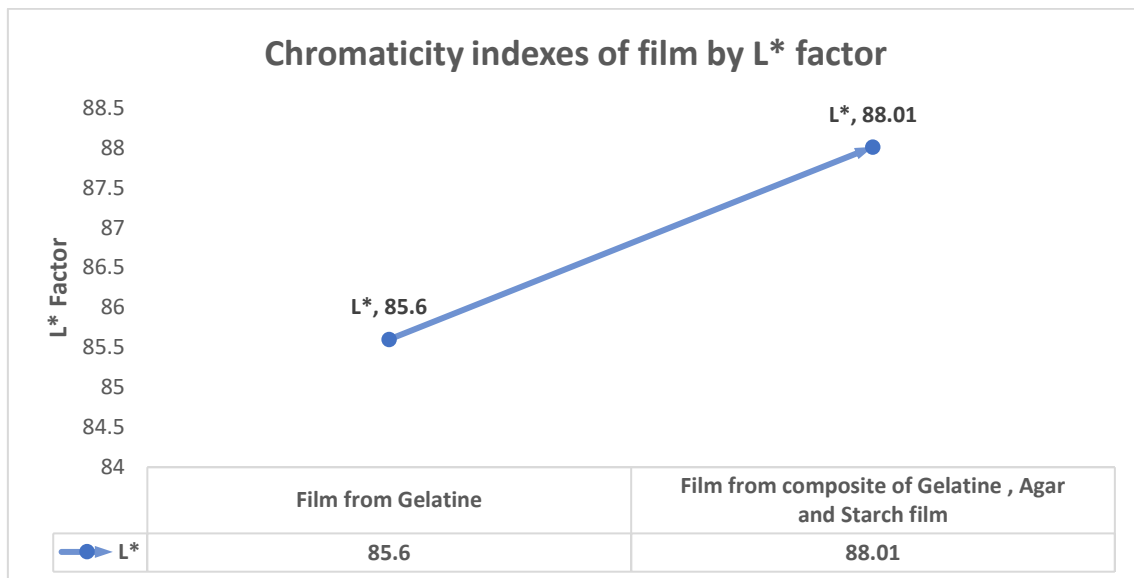


Fig. 5.2 Chromaticity indexes of film by L^* factor

5.3.1.3 CHROMATICITY INDEXES OF FILM BY a^* FACTOR

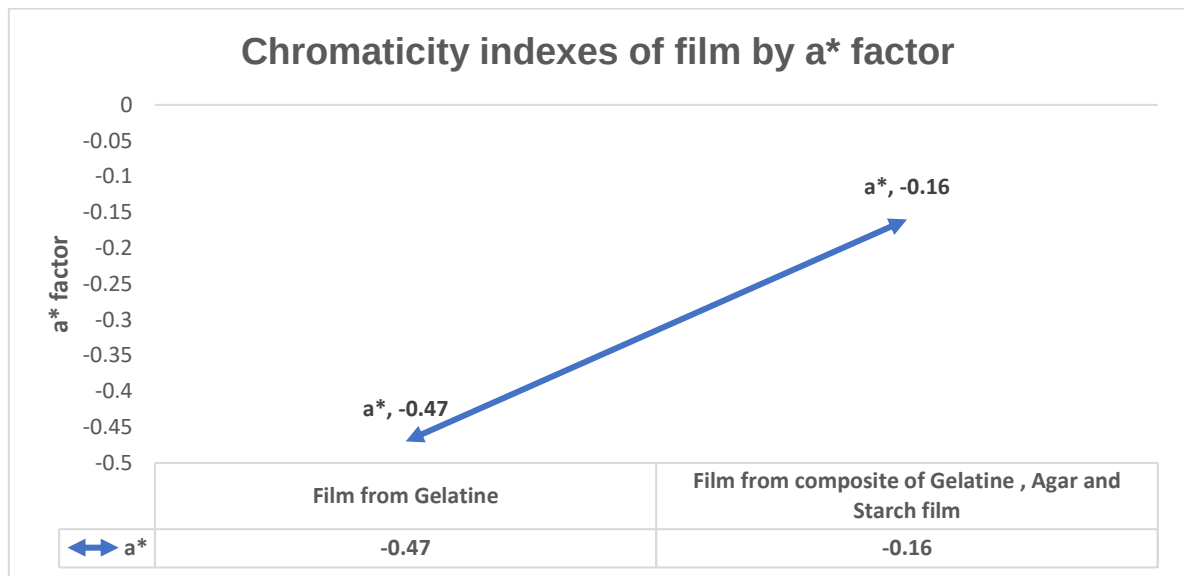


Fig. 5.3 Chromaticity indexes of film by a^* factor

5.3.1.4 Chromaticity indexes of film by b^* factor:

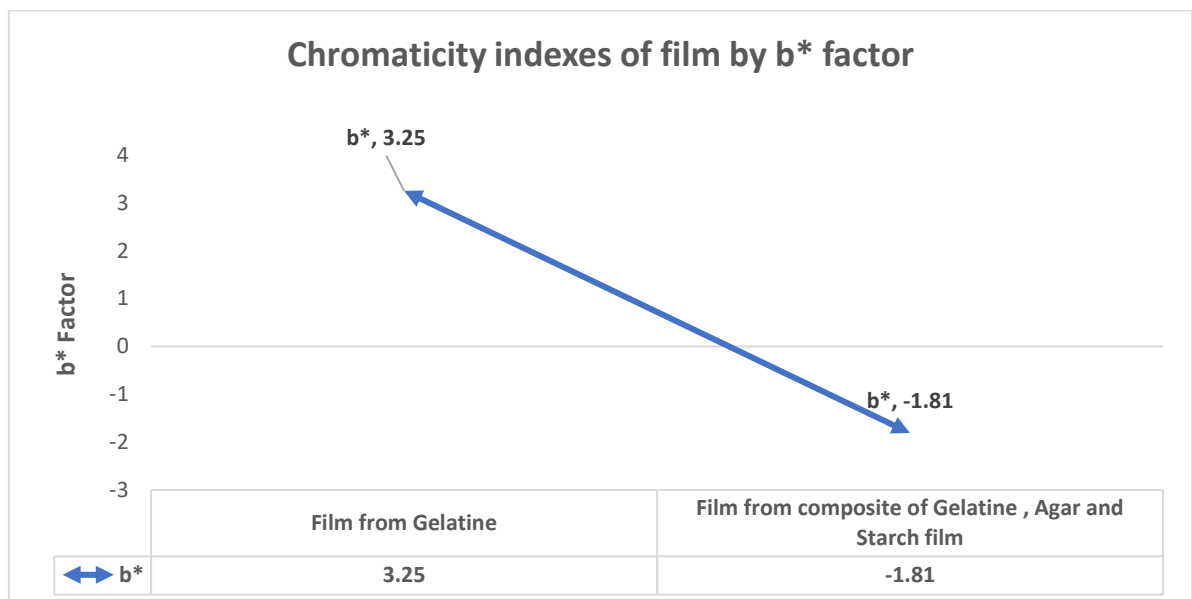


Fig. 5.4 Chromaticity indexes of film by b^* factor

5.3.2 THICKNESS OF FILM

Thickness of an obtained by testing method ASTM D6988 CI 8.4:2013. Thickness of film can be measured by using profilometer or AFM to find the exact thickness. Unit defined as micrometer. In this test, it has been clearly shown on increase in thickness of composite film of gelatine, Agar and starch when compared to other film produced by only Gelatine as a source ingredient.

5.3.2.1 THICKNESS OF FILM PREPARED

Table 5.3 Thickness of Film

Film sample	Thickness	Unit
Film from Gelatine	99.8	μm
Film from composite of Gelatine, Agar and Starch	146	μm

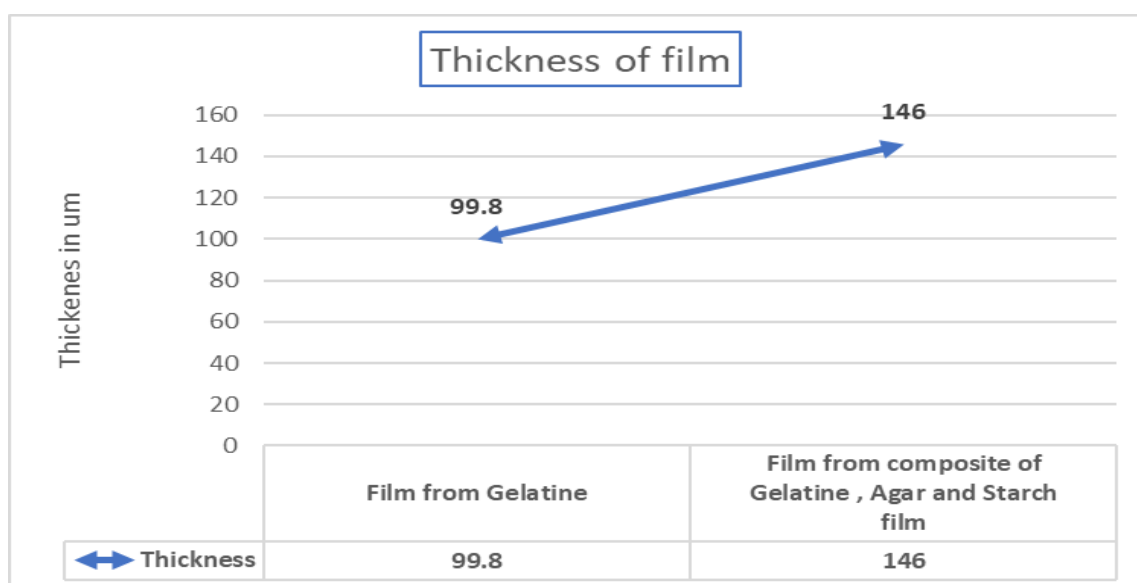


Fig. 5.5 Thickness of Film

5.3.3 COMPOSITIONAL ANALYSIS BY THERMOGRAVITY (TGA)

By observing the weight change that take place as a sample is heated at consistent place, thermogravimetric analysis (TGA), a method of analysis, can be used to evaluate a materials thermal stability and the percentage of volatile components in it.

Thermogravimetry (TGA) is a method for determining how much a sample weight changes as it heated, cooled, or maintained at a constant temperature. its primary function is to describe materials in terms of their composition. Plastic, elastomers, thermosets, mineral compounds, ceramics, and a wide range of analyses in the chemical and pharmaceutical sectors are only a few examples of the application area.

Produced two films has undergone testing of Thermogravimetry analysis under test method ASTM E1131:2014. This TGA analysis will be analyzed by Gas 1 : Ramp 20.00 °C/min to 650.00 °C & Gas 2 : Ramp 20.00 °C/min to 950.00 °C. There

5.3.3.1 FILM FROM GELATIN SOURCE

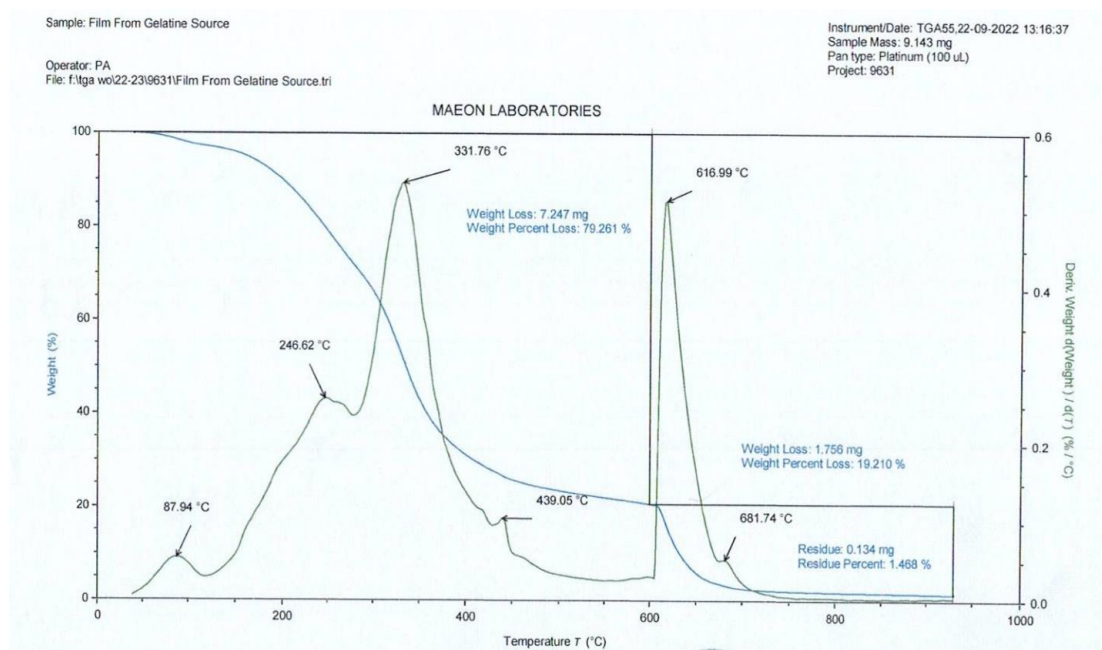


Fig. 5.6 TGA results of Film from Gelatine

Data:

Readings of TGA analysis were given below on analysis of film from Gelatine source.

Table 5.4: TGA Analysis raw data of Film from Gelatine source

Time	Temperature	Weight	Weight	Deriv. Weight
Min	°C	mg	%	% / °C
1.12	38.79	9.14	99.972	-0.00608402
1.17	38.95	9.14	99.971	-0.00610246
2.32	48.05	9.133	99.888	-0.0120847
3.57	67.88	9.091	99.437	-0.0353957
4.62	87.94	9.004	98.48	-0.0547285
4.67	88.9	8.999	98.427	-0.0546941
4.72	89.87	8.994	98.374	-0.0545748
6.07	115.6	8.893	97.265	-0.0299921
7.32	138.86	8.82	96.471	-0.0435341
8.57	162.43	8.684	94.983	-0.0933819
9.82	186.61	8.42	92.096	-0.148041
11.07	211.35	8.024	87.765	-0.194992
12.32	236.47	7.522	82.273	-0.246389
12.37	237.49	7.499	82.023	-0.248509
12.82	246.62	7.286	79.689	-0.259509
13.57	261.95	6.927	75.762	-0.253099
14.82	287.59	6.354	69.502	-0.250861
16.07	313.31	5.574	60.962	-0.443846
16.97	331.76	4.727	51.697	-0.536513
18.57	364.35	3.476	38.016	-0.274709
19.82	389.68	2.991	32.712	-0.161614
21.07	414.88	2.676	29.266	-0.117531
22.27	439.05	2.446	26.751	-0.10561
23.57	465.24	2.28	24.937	-0.0528541
24.82	490.28	2.181	23.855	-0.0357826
26.07	515.53	2.105	23.027	-0.0301853

Table 5.4: TGA Analysis raw data of Film from Gelatine source

Time	Temperature	Weight	Weight	Deriv. Weight
Min	°C	mg	%	% / °C
26.12	516.54	2.103	22.996	-0.0300024
27.32	540.69	2.04	22.312	-0.0266718
28.57	565.83	1.98	21.657	-0.0258584
29.82	590.97	1.916	20.955	-0.0297325
30.92	616.99	1.51	16.51	-0.513416
32.32	662.27	0.413	4.516	-0.0894937
33.17	681.74	0.303	3.316	-0.0525767
34.82	715.39	0.215	2.347	-0.0127721
36.07	739.78	0.195	2.132	-0.00617337
37.32	764.54	0.184	2.009	-0.00460216
38.57	789.56	0.174	1.904	-0.00405212
39.82	814.64	0.166	1.815	-0.00270192
41.07	839.72	0.159	1.743	-0.00276101
42.32	864.78	0.153	1.673	-0.00262604
43.57	889.83	0.147	1.604	-0.00281093
44.82	914.87	0.139	1.521	-0.00354207
44.87	915.87	0.139	1.517	-0.00364219
44.92	916.88	0.138	1.513	-0.00375493
45.02	918.88	0.138	1.506	-0.00388504
45.07	919.88	0.137	1.502	-0.00388094
45.12	920.88	0.137	1.498	-0.00382867
45.32	924.89	0.136	1.483	-0.00365513
45.37	925.89	0.135	1.479	-0.00369616
45.42	926.89	0.135	1.476	-0.00374586
45.47	927.89	0.135	1.472	-0.00376911
45.52	928.89	0.134	1.468	-0.00380188

5.3.3.2 Film from composite of Gelatine, Agar & Starch:

Obtained film was undergone was TGA (Thermo Gravimetry analysis) and results in graph was determined below.

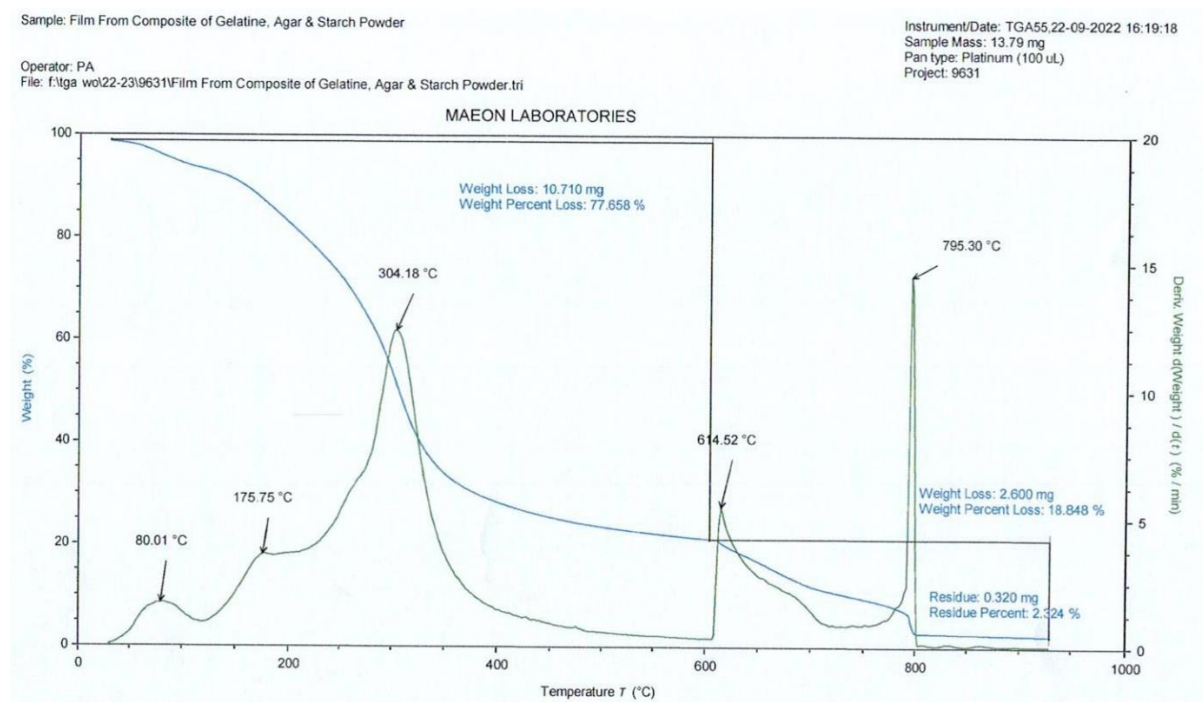


Fig. 5.7 TGA results of Film from composite of Gelatine, Agar and Starch

Data:

Readings of TGA analysis were given below on analysis of film from composite of Gelatine, Agar, and Starch.

Table 5.5: TGA Analysis raw data of Film from composite Gelatin, Agar, and starch source

Time	Temperature	Weight	Weight	Deriv. Weight
Min	°C	Mg	%	% / °C
0.02	29.58	13.633	98.848	-0.098877
0.07	29.59	13.632	98.843	-0.0987396
1.22	29.71	13.617	98.732	-0.0907898
2.47	32.66	13.602	98.625	-0.111725
3.72	45.96	13.563	98.338	-0.434845
4.97	67.54	13.4	97.156	-1.52725
5.62	80.01	13.253	96.095	-1.70966
6.22	91.58	13.115	95.092	-1.59795
7.47	115.2	12.901	93.543	-0.967957
8.72	138.52	12.709	92.152	-1.48268
9.97	162.22	12.34	89.472	-2.92096
10.67	175.75	12.018	87.139	-3.61082
11.22	186.52	11.746	85.166	-3.55542
12.47	211.33	11.122	80.639	-3.68275
12.52	212.34	11.096	80.454	-3.69975
13.72	236.53	10.44	75.701	-4.38231
14.97	262.02	9.538	69.155	-6.20341
16.22	287.69	8.295	60.142	-9.18169
17.02	304.18	7.068	51.248	-12.3879
17.47	313.43	6.325	45.86	-11.2017
18.72	339	4.984	36.141	-4.95043
19.97	364.46	4.368	31.672	-2.63898
21.22	389.73	4.01	29.073	-1.68166
22.47	414.94	3.759	27.258	-1.2749
23.72	440.12	3.563	25.832	-1.03312
24.97	465.35	3.407	24.705	-0.792957

**Table 5.5 TGA Analysis raw data of Film from composite of Gelatine, Agar
and starch source**

Time	Temperature	Weight	Weight	Deriv. Weight
Min	°C	Mg	%	% / °C
26.22	490.44	3.282	23.8	-0.62439
27.47	515.61	3.181	23.067	-0.5373
28.72	540.77	3.095	22.444	-0.462952
29.97	565.91	3.019	21.889	-0.410954
31.22	591.06	2.952	21.403	-0.366978
32.27	614.52	2.82	20.449	-5.49507
33.72	662.03	2.145	15.552	-2.53072
34.97	689.96	1.763	12.782	-1.85543
36.22	714.92	1.532	11.106	-0.950819
37.47	739.33	1.381	10.011	-0.909132
38.72	764.1	1.223	8.869	-0.990797
38.77	765.1	1.216	8.819	-1.00492
39.97	789.14	0.984	7.137	-2.16242
40.27	795.3	0.726	5.261	-14.5258
41.22	814.06	0.393	2.851	-0.100554
42.47	839.29	0.373	2.704	-0.102367
43.72	864.35	0.356	2.578	-0.140603
44.97	889.39	0.341	2.473	-0.0729273
46.22	914.43	0.328	2.379	-0.0741239
46.47	919.44	0.326	2.361	-0.0735588
46.57	921.44	0.325	2.353	-0.072278
46.77	925.45	0.323	2.338	-0.076355
46.87	927.45	0.321	2.331	-0.0740509
46.92	928.45	0.321	2.327	-0.0732355
46.97	929.46	0.32	2.324	-0.0733232

5.3.3.3 COMPARISON ON TGA ANALYSIS OF PRODUCED FILM

a. FILM FROM GELATINE

Obtained film from Gelatine source has been undergone TGA analysis on two parameters, where Ramp 20°C / to 650 °C, weight loss was determined as 7.25mg and weight % were determined as 79.26 %. For the same sample in Ramp 20°C / to 950 °C, weight loss was determined as 1.76 mg and weight % were determined as 19.21% and the residue were obtained and resulted in both ramps as 0.13 mg and 1.47 %

Table 5.6: TGA Results on Film from Gelatine source

TGA Results on Film from Gelatine Source		
TGA	Weight loss (In mg)	Weight % loss
<i>Ramp 20.00 °C/min to 650.00°C</i>	7.25	79.26
<i>Ramp 20.00 °C/min to 950.00°C</i>	1.76	19.21
<i>Residue</i>	0.13	1.47

b. FILM FROM COMPOSITE OF GELATIN, AGAR, AND STARCH

Obtained film from Composite of Gelatine, Agar and Starch source has been undergone TGA analysis on two parameters, where Ramp 20°C / to 650 °C, weight loss was determined as 10.71 mg and weight % were determined as 77.65 %. For the same sample in Ramp 20°C / to 950 °C, weight loss was determined as 2.60 mg and weight % were determined as 18.84% and the residue were obtained and resulted in both ramps as 0.32 mg and 232.4 %

Table 5.7 TGA Results on Film from composite of Gelatin, Agar, and Starch

TGA Results on Film from composite (Gelatine, Agar & Starch) Source		
TGA	Weight loss (In mg)	Weight % loss
<i>Ramp 20.00 °C/min to 650.00°C</i>	10.71	77.658
<i>Ramp 20.00 °C/min to 950.00°C</i>	2.60	18.848
<i>Residue</i>	0.32	232.4

It has been determined that residue will be more in Film obtained from composite source of 232. 4 % when compared to Film from Gelatine source having residue % as 1.47

5.3.4 FTIR FOURIER TRANSFORM INFRARED SPECTROMETER

The Fourier transform infrared spectrometer (FTIR) is a piece of equipment that uses infrared spectroscopy. It is the most modern type of dispersive spectrometer in contrast to others. This is made possible by its excellent precision, accuracy, speed, enhanced sensitivity, ease of use, and sample non-destructiveness. The basis of infrared spectroscopy technology is a molecule's atomic vibrations, which only let it to absorb specific infrared frequencies and energies. The molecules might be recognized and sorted by FTIR since diverse chemicals will have different infrared spectra.

An interferometer is used by the FTIR spectrometer to measure the energy provided to the sample. After the black body emits infrared light, the spectral encoding of signals occurs in the interferometer. The resultant interferogram signal is transmitted or reflected by the sample surface, where certain energy wavelengths

are absorbed. Ultimately, the beam travels through the detector before being transmitted to the computer for processing so that the energy signal can undergo the Fourier transformation.

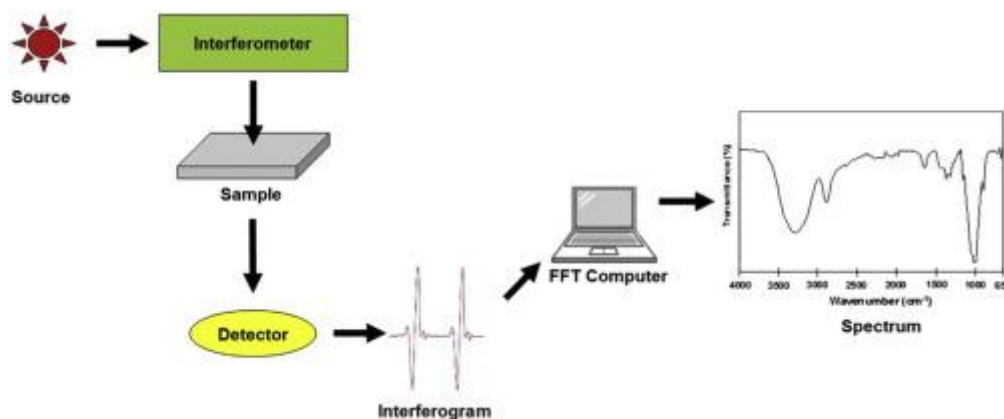


Fig. 5.8 Working principle of FTIR

5.3.4.1 FTIR TEST ON FILM FROM GELATINE SOURCE:

Obtained film from Gelatine source was undergone testing of FTIR (Fourier Transform Infrared Spectrophotometer) under Testing methodology of ASTM E1252: 2021 by using labs.

The Resulted FTIR traces were determined in the environmental condition $23 \pm 2^{\circ}\text{C}$, $50 \pm 5\%$ R. H. Wavelength 600 cm^{-1} to 1500 cm^{-1} will be considered as a fingerprint region whereas 1500 cm^{-1} to 4000 cm^{-1} will be considered as Functional group region. As per traces by FTIR, the peak was observed in between 3200 cm^{-1} to 3400 cm^{-1} which declares that functional group of O-H which has the intensity of Strong and broad.

Second peak in the Functional group region stating that the peak was observed at the range of 1641 cm^{-1} which declares functional group as C = C which has intensity of medium based curve. Third peak in functional group region stating that the peak was observed at the range of 1550 cm^{-1} which declares that functional group of C = N which has the intensity of medium based curve.

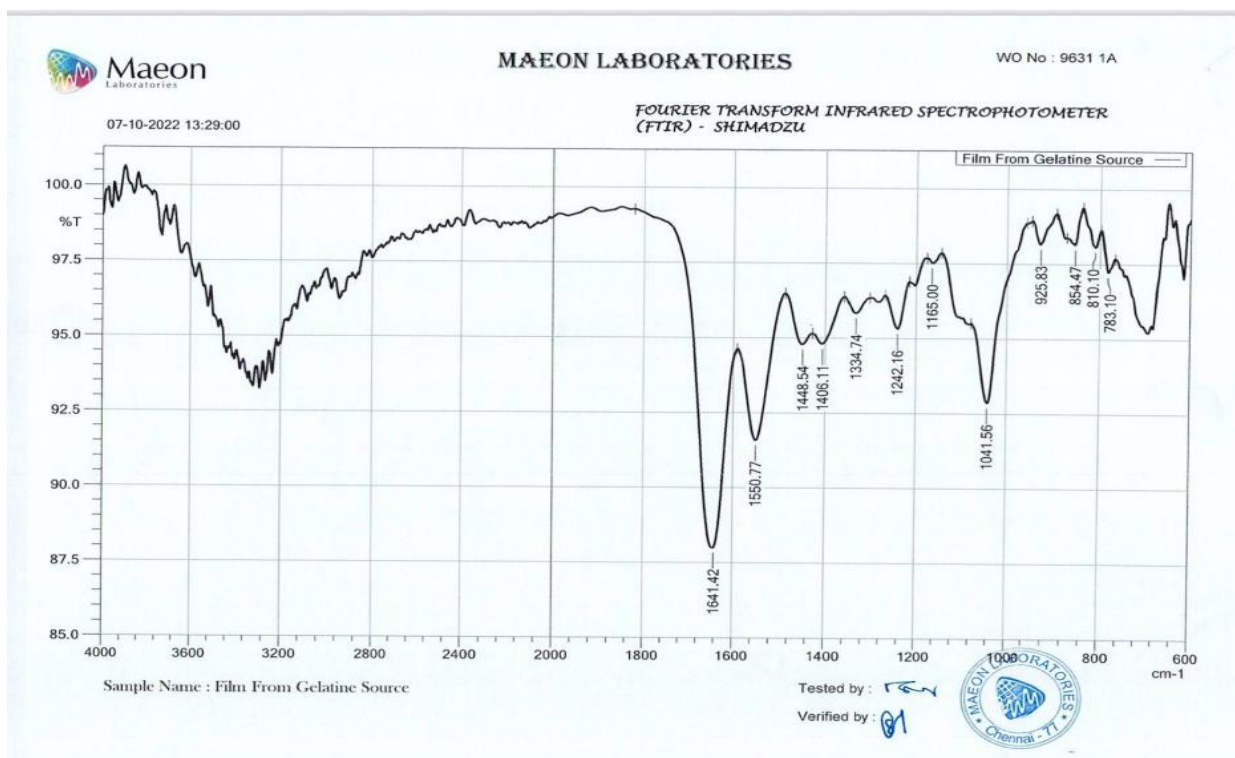


Fig. 5.9 FTIR Results of Film from Gelatine

5.3.4.2 FTIR TEST ON FILM FROM GELATINE, AGAR & STARCH SOURCE

Obtained film from Gelatine source was undergone testing of FTIR (Fourier Transform Infrared Spectrophotometer) under Testing methodology of ASTM E1252: 2021 by using labs. The Resulted FTIR traces were determined in the environmental condition $23 \pm 2^{\circ}\text{C}$, $50 \pm 5\%$ R. H.

The Resulted FTIR traces were determined in the environmental condition $23 \pm 2^{\circ}\text{C}$, $50 \pm 5\%$ R. H. Wavelength 600 cm^{-1} to 1500 cm^{-1} will be considered as a fingerprint region whereas 1500 cm^{-1} to 4000 cm^{-1} will be considered as Functional group region. As per traces by FTIR, the peak was observed in between 3200 cm^{-1} to 3400 cm^{-1} which declares that functional group of O-H which has the intensity of Strong and broad. Another slight peak was detected in the range in

between 2800 cm^{-1} to 3000 cm^{-1} which declares that functional group of C – H which has intensity of medium.

Third peak in the Functional group region stating that the peak was observed at the range of 1651 cm^{-1} which declares functional group as C = C which has intensity of medium based curve. Third peak in functional group region stating that the peak was observed at the range of 1550 cm^{-1} which declares that functional group of C = N which has the intensity of medium based curve

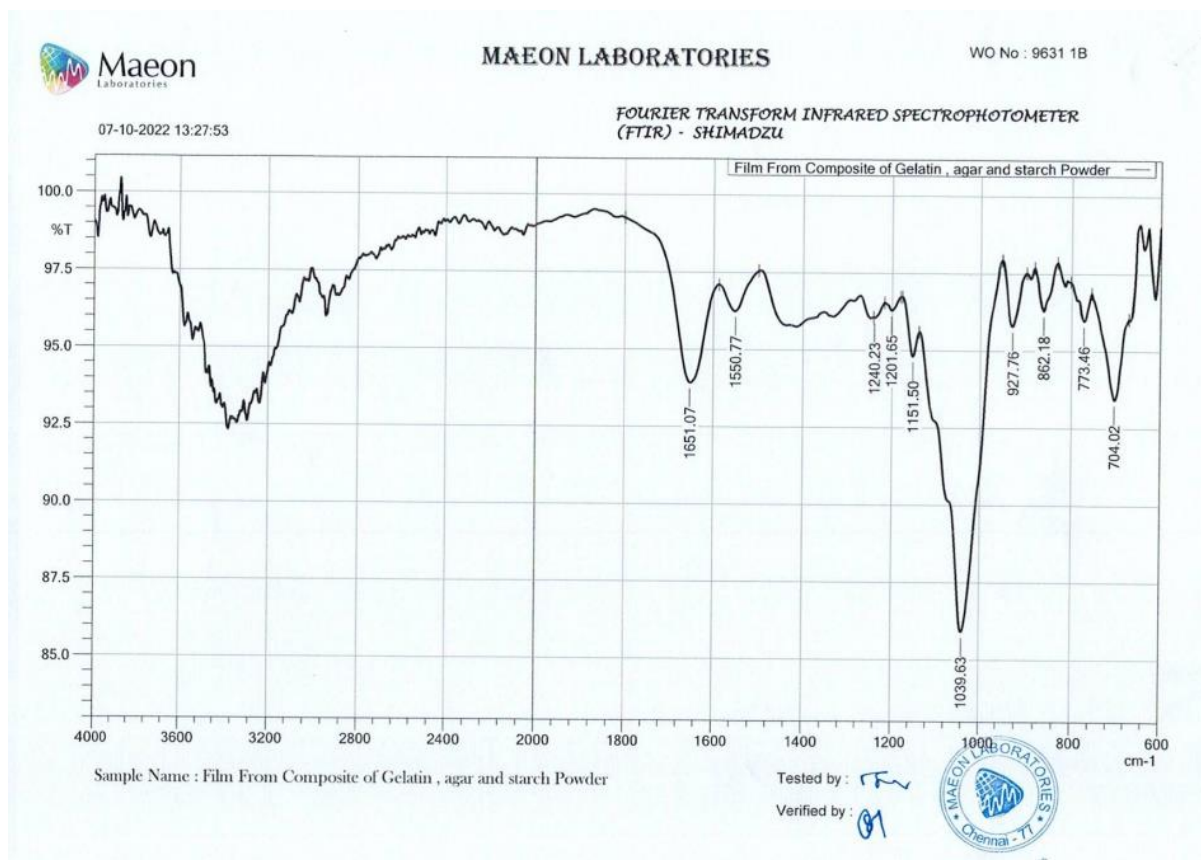


Fig. 5.10 FTIR Results of Film from composite of Gelatine, Agar, and Starch

5.3.5 MICROBIAL TESTING:

The purpose of microbial food testing is to estimate the level of microorganism contamination in raw material and finished goods.

Obtained Film was undergone Swab testing on Several parameters of determine bacterial growth under testing method of APHA 5 Edn 2015 – Chap 5. Below are the parameters tested in the film.

- a. Staphylococcus aureus*
- b. Salmonella*
- c. Escherichia coli*
- d. Total plate count*
- e. Yeast and mould Growth*

a. STAPHYLOCOCCUS AUREUS

One of the most significant bacterial that cause illness to human is staphylococcus aureus. It is the main contributor to cellulitis, abscesses and other skin and soft tissue infection.

b. SALMONELLA

Salmonella infection is the main cause of salmonellosis, a common bacterial illness that affects the intestinal tract. Salmonella bacteria are often eliminated by faeces from both human and animal intestines.

c. ESCHERICHIA COLI

Escherichia coli usually known as E coli is a rod-shaped, Gram negative, facultative anaerobic, coliform bacterium that is frequently found in lower intense

of warm-blooded organism. The majority of E bacteria are benign; however, some serotypes can result in life threatening food poisoning.

Healthy humans and animals generally having Escherichia coli bacteria in their intestines. Vomiting, Bloody diarrhoea and severe stomach cramps are all symptoms of this bacterial infection.

d. TOTAL PLATE COUNT

A microbiological growth medium known as the total plate count analysis method, also known as standard methods, is frequently used to evaluate or track the overall or viable bacterial growth of a sample.

Total plate count (TPC) is a technique for calculating the total amount of bacteria, Yeast, and mould in a substance. The dilution phase of the sample is where the research gets started till it achieves 10^{-5} dilution.

e. YEAST AND MOLD

Yeast are tiny fungi made up of solitary cells that divide by budding. In contrast, moulds develop in long filaments known as hypha that are extended at the apex.

5.3.5.1 SWAB TEST REPORT OF FILM FROM GELATIN SOURCE

Microbial test results of film from gelatine source were tabulated and resulted that there is no microbial growth found in all parameters and now the material is free from microbial growth .

Table 5.8 : Swab test report of film from Gelatine source

MICROBIAL TEST RESULTS OF FILM FROM GELATINE SOURCE		
S,NO	Parameters	Results
01	Staphylococcus aureus	Absent
02	Salmonella	Absent
03	Escherichia coli	Absent
04	Total plate count	< 10 cfu
05	Yeast and Mould	Absent
Note : < 10 cfu can be taken as “ No Microbial growth ” in 1:10 dilution Test method : APHA 5th Edn 2015 – chap 3 CFU : Colony Forming Unit APHA : American public health association		

5.3.5.2 SWAB TEST REPORT OF FILM FROM COMPOSITE OF GELATINE, AGAR & STARCH

Microbial test results of film from gelatine , Agar and starch source were tabulated and resulted that there is no microbial growth found in all parameter and now the material is free from microbial growth .

Table 5.9 : Swab test report of film from composite of Gelatine , Agar, and Starch

MICROBIAL TEST RESULTS OF FILM FROM GELATINE , AGAR, AND STARCH SOURCE		
S,NO	Parameters	Results
01	Staphylococcus aureus	Absent
02	Salmonella	Absent
03	Escherichia coli	Absent
04	Total plate count	< 10 cfu
05	Yeast and Mould	Absent
Note : < 10 cfu can be taken as “ No Microbial growth ” in 1:10 dilution		
Test method : APHA 5th Edn 2015 – chap 3		
CFU : Colony Forming Unit		
APHA : American public health association		

As of results from microbial test, obtained two films were tested and resulted as the sample is free from any bacterial growth and it is fit for use in Food application

5.3.6. APPLICATION OF PRODUCED FILM ON TOMATO

Produced film was applied in experimenting with increase in shelf-life extension of vegetable. For this experiment, Tomato was selected for the application process. The reason behind why tomato has been chosen were illustrated below.

With an annual production of 18.2 million tons, tomatoes are the second largest vegetable crop in India. However, one disadvantages is their shorter shelf life.

After harvest, tomato quality is constantly changing. Tomato fruits ripen during this time, depending on the temperature and harvest maturity, they may soon overripe. The extension of tomato shelf life is crucial for both domestic and international marketing. In general, refrigerated storage increases the shelf life of tomatoes. In refrigerated storage many fruits and vegetables can be kept for several weeks or even months. Low temperature storage controlled atmospheric storage, chemical preservatives, modified atmospheric packing and plastic film wrapping are some of the different storage techniques used to preserve tomato.

5.3.6.1 SELECTION AND CLEANING OF TOMATO

Fully ripened firm fresh tomatoes of local variety were procured from local market. Healthy, defects free tomatoes & fully matured tomato were sorted out and cleaned twice with tap water followed by double distilled water for make tomatoes free from surface contamination. The cleaned tomatoes were stored immediately at different storage conditions based on experimental design.

5.3.6.2 SHELF-LIFE EXTENSION OF TOMOTA

Full Factorial design was carried out through the experiment. Two independent variables such as film from Gelatine and film from composite of Gelatine, Agar & starch powder, and storage temperature. Analysis was carried out at every 3 days regular intervals for 15 days to measure the different response of weight loss, Physical change, and Shelf life of tomato when it is wrapped with film.

The packaging materials selected were Film from Gelatine and Film from composite of Gelatine, Agar & Starch. Each tomato was individually wrapped with two different films.

For this trial, Same size three tomato should be taken and 2 tomato should be wrapped with the two obtained film and remaining 1 tomato should be free from any wrapping and kept in separate area at room temperature. These all tomato should be monitored for every 3 days in changes with color and physical condition of tomato.

Table 5.10 Shelf-life extension of tomato – Results

Shelf-Life extension of Tomato (At Room temperature)		
Tomato kept without film wrapping	Tomato wrapped with Film from Gelatine	Tomato wrapped with Film from Gelatine, Agar and starch
11 Days	15 Days	16 Days

As a result, the shelf life of 16 days was observed when the tomato was wrapped with Film from composite of Gelatine, Agar, and starch whereas 15 days were observed for Film from Gelatine source. The shelf life of unwrapped tomato was only 11 days.

5.6.3.3 ESTIMATION OF WEIGHT LOSS OF TOMATO

For Every three days, the weight loss of tomato samples was measured. First, the samples original weight was determined. Every three days, sample were collected, and their ultimate weight was calculated by using an electronic digital scale with an accuracy of 0.01g. The weight loss of samples was calculated from their beginning and final weights and was then reported as a percentage using the formula below.

$$\text{Weight Loss \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

After analyses, weight loss % were determined and tabulated below. Weight loss % was resulted at highest of 3.2 % at the Day 15.

Table 5.11 Weight loss % of Tomato

WEIGHT LOSS (%)		
AT ROOM TEMPERATURE		
Days	Film from Gelatine	Film from Composite
0	0	0
3	0.8	0.9
6	1.6	1.7
9	2.2	2.3
12	2.7	2.8
15	3.2	3.1

CHAPTER 6

6. CONCLUSION

Film has been obtained from different sources of raw material as Gelatine sources and a new ideology of compositing the source ingredients of Gelatine , Agar and Starch with different ratio. Some of the trails were taken by differing the ratio of ingredients to composite the material for film preparation. Out of 4 trails with different ratio, only equal ratio (1:1:1) of ingredients resulted in obtaining film. Remaining trails with other ratio resulted negative in film preparation. This negative results of obtained film is not suitable to get film of its shape and thickness.

Characterization study was done for obtained two films namely Film from Gelatine source and Film from composite of Gelatine, Agar, and Starch. The Characterization study includes Chromaticity value, Thickness, Compositional analysis by Thermogravimetry (TGA), Identification by infrared spectrophotometry (FTIR) and Microbial testing and Application of Film on Increasing shelf life of tomato.

Colour Chromaticity indexes of film was resulted as for film from Gelatine source as (L^* factor : 85.6), (a^* factor : - 0.47) & (b^* factor : 3.25), whereas for it was resulted as for Film from composite of Gelatine, Agar and starch were determined as (L^* factor : 88.01), (a^* factor : - 0.16) & (b^* factor : - 1.81). It has been noticed that major difference observed in B^* factor between both films. Thickness of the film was tested and determined as 99.8 μm for Film from Gelatine source, whereas 146 μm for Film from composite of Gelatine, Agar, and Starch. Thickness of Film obtained from composite of Gelatine, Agar and starch will be high when compared to Film obtained from Gelatine.

Obtained film from Gelatine source has been undergone TGA analysis on two parameters, where Ramp 20°C / to 650°C, weight loss was determined as 7.25mg and weight % were determined as 79.26 %. For the same sample in Ramp 20°C / to 950 °C, weight loss was determined as 1.76 mg and weight % were determined as 19.21% and the residue were obtained and resulted in both ramps as 0.13 mg and 1.47 %

Obtained film from Composite of Gelatine, Agar and Starch source has been undergone TGA analysis on two parameters, where Ramp 20°C / to 650 °C, weight loss was determined as 10.71 mg and weight % were determined as 77.65 %. For the same sample in Ramp 20°C / to 950 °C, weight loss was determined as 2.60 mg and weight % were determined as 18.84% and the residue were obtained and resulted in both ramps as 0.32 mg and 232.4 %

It has been determined that residue will be more in Film obtained from composite source of 232. 4 % when compared to Film from Gelatine source having residue % as 1.47.

For FTIR analysis, obtained film from Gelatine and composite sources was undergone testing of FTIR (Fourier Transform Infrared Spectrophotometer) under Testing methodology of ASTM E1252: 2021 by using labs. The Resulted FTIR traces were determined in the environmental condition $23 \pm 2^{\circ}\text{C}$, $50 \pm 5\%$ R. H.

As of results derived from microbial testing, obtained two films were tested and resulted as the sample is free from any contaminants and any microbial bacterial growth and it is fit for use in food application.

Application of obtained film was experimented to increase the shelf-life extension of tomato and results were determined. As per results the shelf life of 16 days was observed when the tomato was wrapped with film obtained from composite of Gelatin, Agar, and starch whereas 15 days were observed from film obtained from Gelatine source. The shelf life of unwrapped tomato was derived only as 11 days.

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