

FABRICATION AND CHARACTERIZATION OF EDIBLE ZEIN FILM FOR SUSTAINABLE FOOD PACKAGING

A DISSERTATION

*Submitted in partial fulfillment of the requirements
for the award of the degree of*

MASTER OF TECHNOLOGY
IN
POLYMER TECHNOLOGY

Submitted By
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(2K17/PTE/08)

Under the supervision of
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CANDIDATE'S DECLARATION

I hereby declare that the work presented in the dissertation entitled “**FABRICATION AND CHARACTERIZATION OF EDIBLE ZEIN FILM FOR SUSTAINABLE FOOD PACKAGING**” is original and has been carried out by me in the partial fulfillment of the requirement for the award of the Master of Technology in Polymer Technology in the Department of Applied Chemistry, Delhi Technological University, Delhi – 110042, under the supervision of **Dr. Deenan Santhiya**, Assistant Professor, Department of Applied Chemistry. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the project dissertation title **“FABRICATION AND CHARACTERIZATION OF EDIBLE ZEIN FILM FOR SUSTAINABLE FOOD PACKAGING”** which is submitted by VIKAS KUMAR CHANCHAL, 2K17/PTE/08, Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place:

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Date:

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ACKNOWLEDGEMENT

The success and final outcome of this project required a lot of guidance and assistance from many people and I am extremely fortunate to have got this all along the completion of this project work.

I wish to express my gratitude towards my project supervisor, **Dr. Deenan Santhiya, Department of Applied Chemistry, Delhi Technological University**, who provided me a golden opportunity to work under her able guidance. Her scholastic guidance and sagacious suggestions helped me to complete the project on time.

I wish to thank **Dr. Archana Rani , Professor and Head of the Department of Applied Chemistry, Delhi Technological University**, for her constant motivation and for providing able guidance.

I am thankful to and fortunate enough to get constant encouragement, support and guidance from all teaching as well as non-teaching staffs of Department of Applied Chemistry and Polymer Technology, which helped me in successfully completing my project work. I am also thankful to PhD scholars Himansh Goel, Manjot Kaur, Meenakshi Gautam , Radha Sachan for their constant support and motivation.

Finally, yet importantly, I would like to express my heartfelt thanks to my beloved family and friends who have endured my long working hours and whose motivation kept me going.

Vikas Kumar Chanchal

ABSTRACT

Due to their advantages of using as edible products over synthetic polymers, edible polymers have created significant deliberation in contemporary eons. This could help to reduce the contamination of the environment. Compared to the more traditional non-environmentally friendly plastics, edible polymers can virtually decrease the complexity and thus enhance the recyclability of products and can replace such synthetic polymers. Edible polymers opened a fresh chance to develop films, coatings, pellets and synthetic nanopolymers, especially for medical, agricultural and industrial applications. Edible polymers provide many benefits for drug delivery and tissue engineering. Edible polymer technology enables to create their products more appealing and secure to use by the food industry. New edible materials have been obtained from many natural sources that have been traditionally considered discarded materials. The purpose of this evaluation is to provide a thorough introduction to edible polymers by offering descriptions of their origin, characteristics and prospective uses. In this study properties and characteristics of pure zein film and cross-linked zein film(ZEO) has been compared .

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Fabrication and Characterization of Zein Film for Sustainable Food Packaging
by Vikas Kumar Chanchal

Submission date: 24- Jun- 2019 04:25PM (UT C+0530)

Submission ID: 1146640833

File name: REPORT _By_Vikas_Chanchal.pdf (1.34M)

Word count : 8379

Character count : 51533

Name :Vikas Kumar Chanchal

Roll No: 2k17/PTE/08

M. Tech PTE (2017-2019)

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Due to their advantages of using as edible products over synthetic polymers, edible polymers have created significant deliberation in contemporary cons. This could help to reduce the contamination of the environment. Compared to the more traditional non-environmentally friendly plastics, edible polymers can virtually decrease the complexity and thus enhance the recyclability of products and can replace such synthetic polymers. Edible polymers opened a fresh chance to develop films, coatings, pellets and synthetic nanopolymers, especially for medical, agricultural and industrial applications. Edible polymers provide many benefits for drug delivery and tissue engineering. Edible polymer technology enables to create their products more appealing and secure to use by the food industry. New edible materials have been obtained from many natural sources that have been traditionally considered discarded materials. The purpose of this evaluation is to provide a thorough introduction to edible polymers by offering descriptions of their origin, characteristics and prospective uses.

CHAPTER -1

INTRODUCTION

Increasing the mandate for high-quality, ready-to-eat food products with a long shelf life contributes to the expansion of new processing technologies that ensure that the natural properties and appearance of the product have not changed dramatically. Food wrapping is contributing to a lot of waste in our households, and most of us just accept it.

Edible polymers were the polymeric material that can readily be taken in whole or in portion by humans or reduced animals through the oral cavity and have harmless health effects. In order to maintain product quality and stability, edible polymers can be applied straight to the surface as extra protection. During manufacturing and storage, the conditions imposed on edible polymers were determined by the product's specific properties and modifications in these characteristics. There were several reasons to investigate edible polymers. one was the introduction of fresh categories of food products, such as secure, convenient and high-quality goods. They safeguard food from nutrient loss. In practice, whipped edible coatings. Edible polymer efficiency was determined by their characteristics of mass transport. Technologist develops and formulates implantable drug delivery systems produced from bioresorbable polymers which gradually release accurate amounts of therapeutic agents over time. Polymer drug conjugates were cancer diagnosis and therapy products of nanomedicine. Because of its accurate taste and biodegradability, natural polymers can be an alternative source for packaging growth.

Edible polymers have emerged as a replacement for synthetic plastic for food applications and in latest years, due to their benefits over synthetic polymer, have gained important attention. The primary benefit of edible polymer over traditional synthetics was that they can be eaten with the products. There is no

package to dispose of, and even if the films are not eaten, they could still lead to environmental effluence decrease. The edible polymers were solely manufactured from renewable, edible ingredients and were therefore expected to degrade more easily than other polymeric materials. Edible polymer can expand the organoleptic properties of packaged foods as long as they contain different components such as flavorings, colors and sweeteners. In the food industry and medical sectors, their use based on natural polymers and food grade additives has been steadily increasing. With the addition of plasticizers and surfactants, the edible polymer can be produced with a variety of natural products such as polysaccharides, proteins and lipids. The functionality and efficiency of edible polymer depends primarily on its barrier, mechanical and color characteristics, which in turn depends on the structure of the film and its process of creation.

Food products were usually coated by dipping or spraying, forming a thin film on the food surface that acts as a semi-permeable membrane that in turn controls the loss of moisture or suppresses the transfer of gas. The edible polymer also works as antimicrobial and antioxidant carriers. Edible polymer production creates less waste and pollution, but its permeability and mechanical characteristics were usually lower than synthetic polymer.

Currently around 150 million tonnes of plastic have been generated worldwide annually, and manufacturing and consumption are continuing to rise. Most of these plastics were based on crude oil and any increase in their manufacturing outcomes in increased use of oil, causing severe contamination of the environment owing to wasted and undegraded polymer. It is necessary to consider edible and biodegradable polymers as a replacement for more traditional recycling processes and this has encouraged scientists to synthesize fresh polymers that can be returned to the biological cycle after exercise. Thus, the use of readily biodegradable agricultural polymers would not only fix these issues, but also provide potential fresh uses for surplus farm products. Due to its biodegradability, edible, widely available, abundant, low price, non-allergic, simple to use and thermoprocessable, starch-based material has gained excellent attention in the food packaging sector.

Components used in edible polymer preparing can be categorized into four categories: hydrocolloids, polypeptides, lipids, and composites. Hydrocolloid films have excellent oxygen, carbon dioxide and lipid barrier characteristics, but not water vapor characteristics. Also, most hydrocolloid polymers have superb mechanical characteristics, which have been very helpful for fragile food products.

Among them, the most appealing were protein-based edible polymers. Compared to those made from lipids and polysaccharides, these polymers have remarkable gas barrier characteristics. Some protein-based edible polymers such as rapeseed protein mixed with gelatin (tensile strength—53.45MPa) have had better mechanical characteristics than polysaccharide and fat-based films. Edible polymer based on proteins can form bonds in distinct positions and offer a strong potential for numerous bonds. It seems nice option to plastic foil. They have been described as material that can be eaten, providing a good barrier to food moisture, oxygen, and solvent movements.

Since edible polymers were deemed both a packaging and a food element, a number of necessities should be met, such as high barriers and mechanical efficiencies and biochemical, physicochemical and microbial stability; they should be non-toxic, non-polluting and low-cost.

The films can be applied to the interfaces between distinct layers of elements within heterogeneous foods. They can be customized in products such as pizzas, pies and sweets to avoid deteriorating inter-component humidity and solute migration. The films can be used as antimicrobial and antioxidant carriers. They can also be used on the food surface in a comparable implementation to regulate the rate of diffusion of preservative substances from the ground to the food interior. Another possible application for edible films might be their use together with non-edible films in multilayer food packaging products. In this situation, the edible films would be the inner layers in direct contact with food products. Extensive study on the growth of new products, film formation techniques, techniques for improving film characteristics and prospective applications is required.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Edible Films

Edible films are described as a small coating of fabric that can be eaten and provide the food with a obstacle to moisture, oxygen and solute motion. The material can be a full food coating or a constant layer of food parts can be disposed of (Guilbert, 1986). Edible films can be created as food coatings and free-standing films and can be used as a gas aroma barrier with food (Kester and Fennema, 1986). However, the development of food application films still requires technical data (Donhowe and Fennema, 1993). Due to their benefits over synthetic films, edible films and coatings have gained significant attention in latest years. The primary benefit of edible films over traditional synthetics is that the people can consume them. There is no package to dispose of even if the films are not consumed, they could still contribute to reducing pollution from the environment. The films are made solely from natural, organic ingredients and are thus expected to degrade faster than polymeric materials. Films can improve the organoleptic characteristics of wrapped products as long as they involve different elements (flavorings, colours, sweeteners). The films can be used for personal packaging of tiny parts of meat, especially products that are not presently produced separately for practical purposes such as pears, beans, nuts and strawberries. The films can be used for personal packaging of tiny parts of meat, especially products that are not presently produced separately or practical purposes such as pears, beans, nuts and strawberries. The films can be used for personal packaging of tiny parts of meat, especially products that are not presently produced separately for practical purposes such as pears, beans, nuts and strawberries.

2.2 Need for Edible Films for Packaging

Increased customer supply for improved food performance and freshness has resulted in the notion of effective packaging- a form of packaging that changes eating circumstances to keep product value and freshness, improve cognitive characteristics, or improve product security and shelf lives. New and innovative food-grade product products or techniques have been and proceed to be created to satisfy the increasing requirement for recyclable or organic products and customer requirements for safer and higher value products. Examples of such packaging materials include biobased polymers, bioplastic or organic polymer packaging products produced from agricultural or marine raw materials. Nanocomposite implementation vows to increase the use of organic and biodegradable polymers. It will help decrease in packaging pollution active with processed foods and promote new food preservation by expanding their shelf life. Polymer composites were mixtures of polymers with certain geometries (fibers, flakes, spheres, and particulates) of inorganic or organic additives. The use of nanoscale fillers has resulted in the growth of polymer nanocomposites and is a radical solution to these standard polymer composites.

2.3 Classification of edible films and coatings

Edible films can be made from film-forming products. Film products need to be dispersed and dissolved during manufacturing in a solvent such as air, sugar or air and tobacco combination or a combination of other solvents. Plasticizers, antimicrobial agents, colors or flavors can be added in this process. To promote dispersion, it is possible to adjust the pH and/or heat alternatives for the particular polymer. Film solution is then casted and dried to obtain free-standing films at the desired temperature and relative humidity. Components used in culinary film preparing can be categorized into three classifications: hydrocolloids

(such as proteins, polysaccharides, and alginates), lipids (such as fatty acids, acylglycerol, waxes) and composites (Donhowe and Fennema, 1993).

Polysaccharides

Polysaccharides used in natural films or coatings include cellulose, starch derivatives, pectin components, parts of seaweed, exudate gums, microbial gums and chitosan (Krochta and Mulder-Johnson, 1997). In general, polysaccharides are very hydrophilic, leading to bad characteristics of water vapor and oil barriers. While polysaccharide polymer coatings may not provide a useful obstacle to water vapor, these coatings may function as sacrificing substances that retard meat/food product humidity deterioration (Kester and Fennema, 1986).

a. Cellulose and derivatives

Cellulose consists of recurring units of D-glucose connected by β -1, 4 glycosidic connections. The hydroxymethyl clusters of anhydrous glucose proteins are situated alternately above and below the polymer spine plane in their native state. This ends in a very narrow packaging of polymer chains and an extremely crystalline structure that in aqueous medium resists salvation. Water solubility may be improved by preparing alkali cellulose to expand the composition, accompanied by chloroacetic acid, magnesium chloride or propylene oxidation reactions to carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxypropyl cellulose (HPMC), HPC and CMC have excellent film-forming characteristics; films are usually odorless and tasteless, versatile and moderately strong, clear, oil and fat resistant, water-soluble, mild to humidity (Krochta and Mulder-Johnson, 1997). MC is the most water-resistant and the smallest derivatives of hydrophilic cellulose (Kester and Fennema, 1986), but the water vapor permeability of cellulose ether film remains comparatively low. In deep-frying meat products, oil absorption was delayed (Kester and Fennema, 1986; Balasubramanium et al., 1997). MC could be

used as a obstacle to lipid migration as a layer on confectionery goods (Nelson and Fennema, 1991). A proportion of scientists explored synthetic films consisting of MC or HPMC and different types of proteins such as beeswax and fatty proteins (Kemper and Fennema, 1984; Kester and Fennema, 1986; Greener and Fennema, 1989a; Koelsch and Labuza, 1992; Debeaufort et al., 1993; Park et al., 1994). Many have water vapor permeability similar to polyethylene (LDPE) of small density.

b. Chitin and chitosan

Chitin is the second most common natural biopolymer (after cellulose) discovered in crustacean exoskeletons, fungal tissue membranes and other biological components (Andrady and Xu, 1997). It is primarily poly (β -(1-4)-2-acetamide-D-glucose), which is structurally identical to cellulose except that secondary hydroxyl is replaced by an acetamide group on the second carbon atom of the hexose repeat unit. Chitosan in the existence of alkali is obtained from chitin by deacetylation. Chitosan is therefore a copolymer composed of β -(1-4)-2-acetamido-D-glucose and β -(1-4)-2-acetamide-D-glucose components, the latter generally reaching 80%. Chitosans are defined in words of degree of deacetylation and median molecular weight and their significance lies in their antimicrobial characteristics together with their cationicity and forming characteristics (Muzzarelli, 1996). Chitosan can create semi-permeable coatings that can alter the inner atmosphere, stopping ripening and lowering fruit and vegetable transpiration levels. Films from aqueous chitosan are clear, tough, flexible and good oxygen barriers (Sandford, 1989; Kalplan et al., 1993). Methylation of polymers could improve the permeability of carbon dioxide. Butler et al. (1996) noted that chitosan films were fairly stable and only mildly altered their mechanical and barrier characteristics during retention. Chitosan coatings are usually used on fruit and vegetable products such as strawberries, cucumbers, bell peppers as antimicrobial

coating (El Ghaouth et al., 1991a, 1991b), and on apples, pears, peaches and plums as gas barrier (Elson and Hayes, 1985; Davies et al., 1989).

c. Starch

Starch is a polymer carbohydrate consisting of blocks of anhydrous glucose. This is not a standardized substance and there are two kinds of glucose polymers in most starches: a linear fiber molecule called amylose and a twisted glucose polymer called amylopectin (Rodriguez et al., 2006). Starches are frequently used in agricultural ingredients. Because of their small price and renewable capacity, they have been used to create biodegradable films to partly or completely substitute plastic polymers and have excellent mechanical characteristics (Xu et al., 2005). High amylose starch like maize starch is a good source of film formation, free-standing films can be made from aqueous amylose gelatinized solution and drying. Normal maize starch is made up of about 25% amylose and 75% amylopectin. Mutant maize differs that comprise up to 85% amylose starch (Whistler and Daniel, 1985). Mark et al. (1966) noted that films generated from elevated amylose maize starch (71% amylose) had no detectable permeability of air at RH concentrations below 100%. For both unplasticized and plasticized (16% glycerol) films, this was accurate. This outcome is unexpected given that adding plasticizers and absorbing water molecules through hydrophilic polymers increases the flexibility of the polymer chain and usually leads to enhanced oxygen permeability (Banker et al., 2000).

Lipid films

Lipid compounds used as safety layer composed of acetylated mono-glycerides, artificial resin and surfactants. Paraffin wax and beeswax are the most efficient lipid ingredients. Because of their relatively small polarity, the primary role of a lipid layer is to prevent moisture movement. The hydrophobic feature of lipid, on the other hand, shapes thinner and more brittle films. As a result, film forming inhibitors such as proteins or cellulose derivatives must be connected with

1 them (Debeaufort et al., 1993). Water vapor permeability generally decreases as the hydrophobicity stage level rises. A polymer framework matrix often supports lipid-based films,

1 a. Waxes and paraffin

Paraffin wax is obtained from crude petroleum distillate portion and is a combination of strong hydrocarbon produced by ethylene catalytic polymerization. The use of paraffin wax on cooked fruit and vegetables and cheese is allowed. Carnauba wax (Copaernica cerifera) is an exudate from palm tree leaf. Beewax (black wax) from honeybees is generated . Candelilla is produced from the plant of candelilla. A combination of liquid paraffin and naphtheric hydrocarbon comprises of mineral water (Hernandez, 1994). Waxes are used as oil and humidity obstacle films (new fruit peel) and to enhance the presence of different products on the ground. When implemented as a dense coating, they must be separated before consumption (some pie) ; they are regarded usable when used in small parts. Waxes (paraphine, carnauba, candellila and mouse glue in particular) are the most effective medicinal compounds that provide a barrier to humidity.

b. Acetoglyceride

Glycerol monostery acetylation produces 1-stearodiacetine by its response to acetic anhydride. This acetylated monoglyceride displays the unique characteristic of solidifying from the molten state into a flexible, wax-like solid (Feuge et al., 1953). Most lipids in the solid state can be stretched to only about 102% of their original length before fracturing. Acetylated glycerol monostearate, however, can be stretched up to 800% of its original length (Jackson and Lutton, 1952), water vapor permeability of this film is much less than that of polysaccharide films with the exception of methyl cellulose or ethyl cellulose (Kester and Fennema, 1986). Acetylated monoglyceride coatings have been used on poultry and meat cuts to retard moisture loss during storage (Kester and Fennema, 1986).

c. Shellac resins

Shellac resins are a secretion of the *Laccifer lacca* insect and consist of a complicated blend of aliphatic polymers of hydroxyl alcohol. In alcohols and alkaline alternatives, this resin is flexible. Shellac is not a GRAS substance; it is only allowed in food coatings and adhesives as an indirect food additive. It is mostly used in pharmaceutical industry coatings and only few food studies have been published (Hernandez, 1994). Rosins which are obtained from the oleoresins of the pine tree are residues left after distillation of volatiles from the crude resin. Resin and its derivatives are widely used in coating for citrus and other fruits (Hagenmaier and Baker, 1993). These coatings were designed primarily to impart high-gloss at the time of inspection by the buyer, usually after application of the coating. When coatings are applied to fruit, they form an additional barrier through which gases must pass. Because coatings differ in gas permeance and ability to block openings in the peel, they have different effects on gas exchange (Hagenmaier and Baker, 1993). In general, citrus fruits with shellac and wood-based resin coatings have reduced inner O_2 , greater inner CO_2 and greater ethanol output than wax-coated crops (Hagenmaier and Baker, 1993). In effect, high ethanol consumption is a sign of off-flavor (Ahmad and Khan, 1987; Cohen et al., 1990; Ke and Kader, 1990). Coatings based on shellac and wood resin also appear to boost the incidence of pitting after planting (Petracek et al., 1997, 1998).

Protein films

Proteins usually occur in their native states as either fibrous proteins that are water-insoluble and act as the primary structural materials of animal tissues, or

globular proteins that are water-soluble or aqueous alternatives of acids, roots or salts and that operate extensively in human structures (Scope, 1994). In simultaneous constructions, fibrous proteins are fully expanded and tightly linked to shape polymers, usually through hydrogen bonding. Combining hydrogen, ionic, hydrophobic and covalent (disulfide) connections, globular proteins bend into complex spherical constructions (Scope, 1994). These polymers ' chemical and physical characteristics rely on the comparative quantities of the components of the amino acid element and their positioning along the polymer protein loop. Several globular proteins have been explored for their film characteristics, including wheat gluten, maize zein, soy protein, and whey protein. Protein films are usually created as the solvent / carrier evaporates from dna alternatives or dispersions. Solvent / carrier is usually restricted to mixtures of air, oil or ethanol-water (Kester and Fennema, 1986). Proteins generally need to be denatured by temperature, acid, foundation, and/or liquid to create the larger structures needed to create films. Protein strands may be associated by hydrogen, ionic, hydrophobic, and covalent bonding once linked. The degree of neck expansion and the type and structure of amino acid residues affect the chain-to-chain relationship that generates cohesive films. Uniform distribution along the polymer chain of polar, hydrophobic, and/or thiol units increases the probability of the corresponding relationships. Increased reactions between polymer chain and chain lead to films that are heavier but less versatile and less permeable to gases, vapors and liquids (Kester and Fennema, 1986).Polymers that contain organizations that can be associated by hydrogen or ionic bonding consequence in films that are great obstacles to oxygen but prone to humidity (Salame, 1986). Therefore, protein films at high relative humidity are anticipated to be excellent oxygen barriers. Different kinds of protein were used as edible films. These include gelatin, casein, whey protein, maize zein, grain gluten, milk protein, fruit mung protein, and peanut protein (Gennadois et al., 1993; Bourtoom, 2008).

a. Gelatin films

Gelatin is acquired through regulated hydrolysis from the insoluble fibrous protein, collagen, which is commonly discovered in existence as the main component of skin, muscles and connective bodies. Gelatin is composed of a unique sequence of amino acids. The characteristic features of gelatin are high content of the amino acids glycine, proline and hydroxyproline. Gelatin also has a mixture of single and double unfolded chains of hydrophilic character (Ross, 1987). Gelatin aqueous solutions are in the sol state at about 40°C and on strains they make physical, thermoreversible gels. The structures experience a conformation disorder-order transformation during gelation and appear to restore the triple-helix framework of collagen (Ross-Murphy, 1992). Gelatin films could be created from 20-30% gelatin, 10-30% plasticizer (glycerin or sorbitol) and 40-70% air accompanied by gelatin gel processing (Guilbert, 1986). Gelatin is used to encapsulate meat and pharmaceutical components in the small humidity or petroleum stage. Such encapsulation provides protection against oxygen and light and defines the quantity of ingredients or the dosage of drugs (Gennadios et al., 1994). In addition, gelatin films have been formed as coatings on meats to reduce oxygen, moisture and oil transport (Gennadios *et al.*, 1994). The most significant protein in maize is Zein. It is a component of prolamin and dissolves in 70–80% oil (Dickey and Parris, 2001, 2002; Landry, 1997). Zein is a fabric that is comparatively hydrophobic and thermoplastic. Zein's hydrophobic shape is associated with its elevated non-polar amino acid material (Shukla and Cheryan, 2001). Zein has great characteristics of film forming and can be used to make biodegradable films. By developing hydrophobic, hydrogen and restricted disulfide bonds between zein strands, the zein bio-film is created (Guilbert, 1986). Dry aqueous ethanol remedy of zein can form edible films (Gennadios and Weller, 1990). Film formation is thought to require the growth of hydrophobic, oxygen and restricted disulfide bonds in the film matrix between zein strands (Gennadios et al., 1994). The subsequent films are fragile and thus involve additional plasticizer to increase stability (Park, 1991). Compared to other culinary films, Zein films are

comparatively excellent air vapor obstacles (Guilbert, 1986). By taking fatty acids or using a cross-linking reagent, the characteristics of the water vapor obstacle can be enhanced. But the edibility of these films is of interest when cross-linking antibodies are used (Alikonis, 1979). Zein layer also demonstrated the capacity to decrease reduction of moisture and firmness and to prevent colour shift (decrease oxygen and carbon dioxide absorption) in new apples (Park et al., 1994b).

c. Wheat gluten films

Wheat gluten is a particular word for wheat flour water-insoluble proteins consisting of a combination of polypeptide molecules that are deemed globular proteins. Gluten cohesiveness and elasticity offer wheat dough stability and promote the development of films. Wheat gluten includes the wheat flour protein prolamine and glutelin fractions, typically referred to as gliadin and glutenin. Although gliadin is dispersed in 70% oil, glutenin is not present (Gennadios and Weller, 1990). While in artificial water insoluble, wheat gluten dissolves at small ionic strength in aqueous solutions of elevated or small pH (Krull and Inglett, 1971). Dry aqueous ethanol mixture of wheat gluten can form edible films (Gennadios and Weller, 1990). In addition to hydrogen and hydrophobic bonds, the cleavage of native disulfide bonds during heating of film-forming solutions and the formation of new disulfide bonds during film drying is considered important for the formation of wheat gluten film structure (Gennadios and Weller, 1990). In order to enhance film stability, it is essential to add plasticizer such as glycerin in gluten films (Gennadios et al., 1994). However, growing film stability by raising the material of sorbitol lowers the characteristics of film resistance, elasticity and air gas barriers (Gontard et al., 1992). The impact of wheat gluten quality on film image and mechanical characteristics was verified by Gennadios and Weller (1992), i.e. higher quality of gluten outcomes in heavier and better films. Herald et al. (1995) studied the impact of bread gluten plasticizer volume; spray-dried fruit gluten films were harder than flash-dried films

with bigger droplets. The egg value was preserved for 30 days when used as a layer on grade A-quality shell eggs. Gluten film tensile strength can be enhanced by using a cross-linking agent such as glutaraldehyde or 80°C heat curing drug (Gennadios and Weller, 1992; Koelsch et al., 1992). The impact of comparative moisture on coal dioxide sorption in grain gluten films was determined by Pochat-Bohatier et al. (2005). The findings indicated that, with the increase in RH, the permeability was dependent on the increase in Co₂ solubility and diffusivity. The rise in wheat gluten's fluid quality increases the bond between carbon dioxide and protein matrix, resulting in elevated RH sorption scores.

d. Protein soy films

Soybean protein content (38-44%) is much greater than fruit seed protein material (8-15%). Most soybean dna is water-insoluble but safe in dilute solid salt alternatives. Soy protein therefore refers to the category of globulin (Kinsella, 1979). Soy protein is globular in shape and is further categorized according to comparative sedimentation rates into 2S, 7S, 11S and 15S fractions (Gennadios et al., 1994). The main elements are the fractions 7S (conglycinin) and 11S (glycinin), both having a quaternary (intermediate) composition (Kinsella et al., 1985). Protein soy is rich in concentrations of asparagine and glutamine. Tightly formed proteins are conglycinine and glycinine. While the amount of conglycinin disulfide cross-linking is restricted owing to just two to three cysteine clusters per molecule, there are 20 intramolecular disulfide bonds in glycinine (Kinsella, 1979). Alkali and boiling both trigger dissociation owing to disulfide bond cleavage and later unfolding of glycinin (Kinsella, 1979). Soy protein-based edible films can be generated in either way: ground film forming on hot soy milk or film forming from soy protein isolate (SPI) alternatives (Gennadios and Weller, 1992). Soy milk is generated by stirring soybeans with water and then separating milk from soybeans obtained. The formation of films from both soymilk and SPI, (a) the heating of film solutions to disrupt the protein structure, the

splitting of native disulfide bonds and the exposure of sulfhydryl groups and hydrophobic groups, and (b) the formation of new disulfide, hydrophobic and hydrogen bonds during film drying are considered important for soy protein film structure (Gennadios et al., 1994; Subirade et al., 1994). Soy protein use has been researched in the creation of films or coatings on meat products (Baker et al., 1994; Gennadios et al., 1994; Stuchell and Krochta, 1994; Kunte et al., 1994). Edible films and coatings in existence can be heterogeneous, composed of a mixture of polysaccharides, proteins and/or lipids. This strategy allows one to use the separate operational features of each previous film category (Kester and Fennema, 1986). The combination of polymers to form films may consist of proteins and carbohydrates, proteins and lipids, carbohydrates and lipids, or synthetic polymers and natural polymers. The main goal of producing composite films is to improve the permeability or mechanical properties as dictated by the need for a specific application. These heterogeneous films are implemented either as an emulsion, suspended, or dispersion of the non-miscible components, or in consecutive strands (multilayer layer or films), or as a remedy in a prevalent solvent. The implementation technique impacts the obstacle characteristics of the acquired films (Guilbert, 1986). The emulsion films from methyl cellulose and fatty acids were launched by Kamper and Fennema (1984) to enhance cellulose film water vapor barriers. The design of composite films centered on the job of Kamper and Fennema (1984) has been widely studied by many scientists recently. Examples of these studies are using lipid and hydroxypropyl methyl cellulose (Hagenmaier and Shaw, 1990), methyl cellulose (MC) and lipid (Greener and Fennema, 1989), MC and fatty acid (Sapru and Labuza, 1994), corn zein, MC and fatty acid (Park *et al.*, 1996), whey isolate and lipids (McHugh and Krochta, 1994), casein and lipids (Avena-Bistillos, 1993), gelatin and soluble starch (Arvanitoyannis *et al.*, 1997), hydroxypropyl starch and gelatin (Arvanitoyannis *et al.*, 1998), corn zein and corn starch (Ryu *et al.*, 2002),

gelatin and fatty acid (Bertan *et al.*, 2005), soy protein isolate and gelatin (Cao *et al.*, 2007), soy protein isolate and polylactic acid (Rhim *et al.*, 2007)

2.4 ZEIN

Zein proteins are the main maize holding cells (Shukla *et al.*, 2001), arranged in endosperm-based cell organs (Cabra *et al.*, 2007). Zein proteins act as a cause of nitrogen for embryos during germination, compromising approximately 45-50 percent of complete maize dna (Shukla *et al.*, 2001) and 44-79 percent of endosperm proteins (Lawton, 2002). Typically, Zein is separated from maize gluten dinner (CGM), a protein-rich by-product of starch production by wet milling by isopropanol solvent extraction. Zein has several exciting characteristics, which could make it a useful option to be used as a pharmaceutical excipient. First of all, zein can create hard, shiny and hydrophobic coatings immune to microbial attack. Second, it is contained in alternatives of aqueous alcohol but not in air (Shukla *et al.*, 2001), meaning its use as a prospective car for regulated substance discharge (Georget *et al.*, 2008). It is also biodegradable and biocompatible (Dong *et al.*, 2004) and possesses powerful adhesive characteristics (Kim, 2008). Zein has indeed been commonly used in the food industry, including packaging and coating, but little is understood about its use in pharmaceutical products

Characteristics of Zein

Prolamins are the composition of Zein proteins, meaning a protein wealthy in proline and glutamine and/or asparagine (Esen, 1986). Zein is especially wealthy in hydrophobic amino proteins (more than 50%), particularly aliphatic amino #oids, with the largest concentrations of leucine (20%), proline (10%) and alanine (10%) (Gianazza *et al.*, 1977; Shukla *et al.*, 2001; Cabra *et al.*, 2006). High aliphatic indexes and elevated ground hydrophobicity reflect this framework (Cabra *et al.*, 2005). Zein has a deficiency in aromatic and fundamental amino proteins but is rich in glutamic acid (21-26%) (Shukla *et al.*, 2001); however, nearly all glutamic and aspartic acid components (glutamines and aspargines, respectively) are amidated (Righetti *et al.*, 1977; Cabra *et al.*, 2006). The constitution of zein

amino acids indicates small polar quantities and a large percentage of nonpolar amino acids that explain their elevated water insolubility and their inclination to aggregate. It also refers to the small volume of aromatic acids and the lack of inhibitors of tryptophan and lysine. Zein's adverse nitrogen balance is explained by the latter function. Being a hydrophobic protein, Zein is regarded (Cabra et al., 2006). A range of scales were created to determine protein hydrophobicity. The hydrophobicity scale of Goldman-Engelman-Steitz (GES) classifies amino acids based on the transition of water-oil free energy from hydrophobic and hydrophilic parts in the amino tissue end shells (Engelman et al., 1986). 76.25% of 3 zein amino plant components are hydrophobic, with the largest concentration assigned to alanine, leucine, proline and serine, according to the GES scale. The aliphatic coefficient that relates to the comparative quantity occupied by the aliphatic end strands can also describe the extremely hydrophobic personality of zein. The Great Hydropathicity Index (GRAVY) classifies proteins according to their water solubility; hydrophobic cells have favorable characteristics while adverse GRAVY scores are shown by hydrophilic proteins. Zein's amino acid sequence produces a elevated aliphatic coefficient (119.3) and a favorable elevated hydropathicity median (0.27). It is also possible to determine the comparative hydrophobicity of zein by calculating its free hydration energy dependent on its amino acid sequence. The hydration-free power calculated for zein is less positive, suggesting greater hydrophobicity compared to albumins and globulins (Cabra et al., 2006). However, despite the presence of hydrophobic and uncharged amino acids, the zein framework includes both hydrophobic and hydrophilic regions that allow it to act as a polymer amphiphile (Wang et al., 2008). Therefore, Zein is prepared to self-assemble as regulated by the system's hydrophilic-hydrophobic equilibrium (HLB) into several mesophases. Zein's shrinking behavior and air sorption (Beck et al., 1996; oh et al., 2003) indicate some protein-water interaction that is usually inclined by the polar end strands of protein fragments of amino acids that make the adsorbed water molecules much attractive (Pauling, 1945).

Zein is a biological combination of distinct molecular volume, solubility and load peptides (Shukla et al., 2001; Lawton, 2002). There are a big range of protein fractions recognized by several writers using different techniques that have generated uncertainty in the zein polypeptide classification. Gianazza et al. (1977) divided zein into two broad fractions, one soluble in alcohol (named Z1) and the other soluble in alcohol containing a reduction agent (Z2), which were further divided into four polypeptide chains of 23 (Z23), 21 (Z21), 13.5 (Z13.5) and 9.6 (Z9.6) molecular weights (kilo Daltons). The last two elements are available only in the Z2 portion and are wealthy in amino acids containing sulfur and are therefore believed to be accountable for the creation of zein granules via disulphide bridges. Esen (1986) divided whole zein into three distinct fractions by variable solubility in liquids comprising between 0 and 95% isopropyl oil (IPA), with or without reduction of antibodies and/or buffers. These three fractions are α -zein, β -zein, and π -zein, respectively. α -Zein was a 50 to 95 percent IPA soluble component, but a 30 percent IPA/30 mM Na-acetate insoluble mixture. β -Zein was the dispersed portion of 30% to 95% IPA alternatives comprising a reduction inhibitor, But both 90% IPA (without reduction product) and 30% IPA/30 mM Na-acetate are insoluble. Soluble in 0 to 80% IPA in the existence of a reduction drug and dissolved in 30% IPA/30 mM Na-acetate. Wilson (1991) separated zein into six classes using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). These classes are called A and B zeins (equivalent to α or 22 and 19 kD zeins, respectively), C zein (β or 14-15 kD zein), D zein (δ or 10 kD zein), E (γ or 27 kD zein) and F (γ or 16 kD zein). In short, zeins can be classified into zeins based on their solubility and molecular weight (Esen, 1986; Wilson, 1991) based on their solubility and molecular weight. α -Zein represents 75-85% of the complete zein and is split into two kinds: Z19 and Z22 of estimated 19 and 22 kDa weights, respectively. β -Zein includes two polypeptides rich in methionine 17-18 kDa, representing 10-15% of the complete zein. The next most common zein (about

20% of the complete zein) (Lawton, 2002) includes two 27 and 16 kDa proline-rich polypeptides. It has an estimated weight of 10 kDa and is a small proportion of the complete zein.

Zein Structure

The primary structure of α -zein, i.e. the amino acid sequence, was derived from chromatography on the zein itself and from the determination of the protein-coding dna (Lai et al., 1999). Z19 and Z22 are composed simultaneously of 210 and 245 amino acids. Both of these zein fractions have sequence homology where non-repetitive N-(of about 10 residues) and C-terminal sequences (36-37 residues) are separated by 9 or 10 domains of repetitive sequences of about an average of 20 residues in Z19 and Z22 zeins, respectively (Tatham et al., 1993). The first proteins to be researched using physical and chemical techniques were α -Zeins (Tatham et al., 1993; Forato et al.). Ultracentrifugation, viscometry, birefringence, and dielectric constant research explored the conformation of zein in alcoholic alternatives. In accordance with the recent SAXS (small-angle X-ray dispersal) tests (Tatham et al., 1993; Matsushima et al., 1997), all previous trials proposed that α -zeins act as asymmetric electrons in equilibrium. In addition, those previous surveys showed extended molecular structures with elevated axial proportions that differ by author and technique.

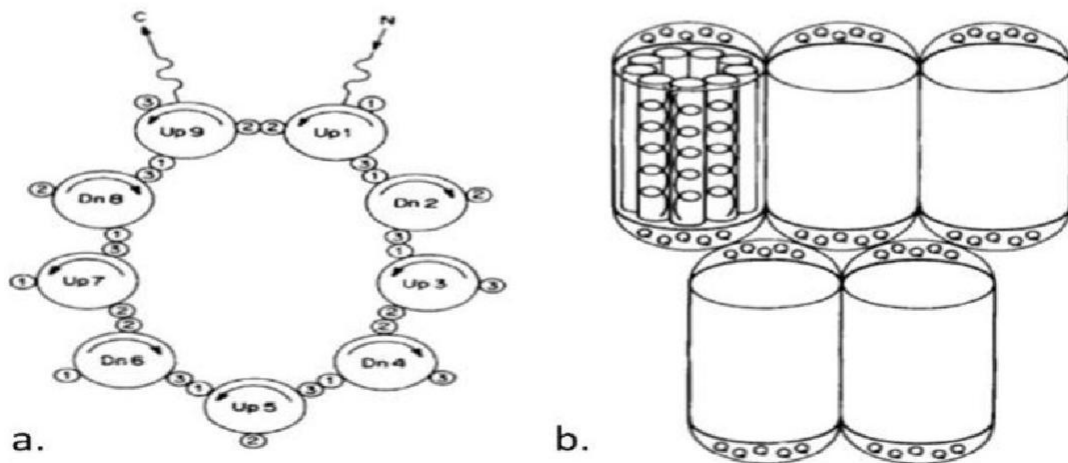


Fig-1 A possible nine-helical zein protein structural model (a) and a possible model for the arrangement of zein proteins within a plane as well as for the stacking of molecular planes (b) (Argos et al., 1982).

A three-dimensional structure of α -zeins has not yet been created by elevated precision methods such as NMR and X-ray diffraction. Several designs were proposed, though. The Argos system (Argos et al., 1982) is one of the constructions earlier suggested. These writers proposed a structural model consisting of nine topologically antiparallel and adjoining helices clustered together with the helical and cylindrical cores placed within a twisted rectangular cross-section cylinder. Intramolecular hydrogen bonding held by the two polar sections of the predominantly hydrophobic helical wheel strengthened the framework. The structure of a zein molecular curve is created through the intermolecular bonding of the helical wheel's fifth linear section and the connection of glutamine acids in bend areas that occur at the cylindrical cluster caps or finishes.

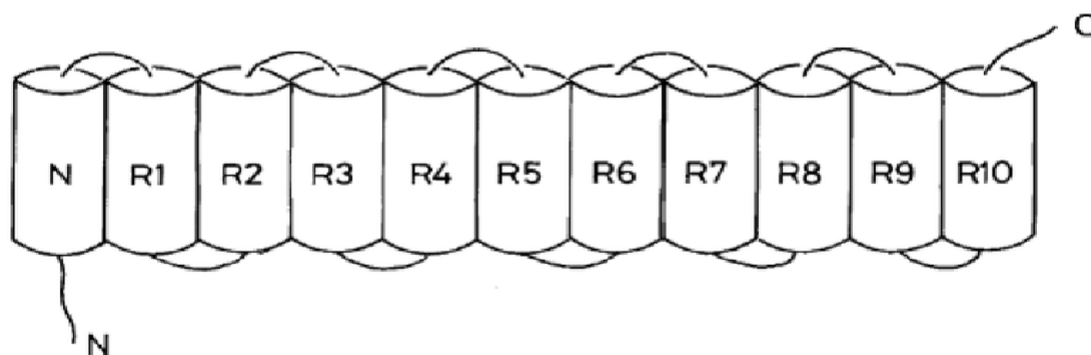


Fig 2 A possible structural model of α -zein (Z22). Each cylinder represents a tandem repeat unit formed by a single α -helix and the glutamine rich turns or loops joining them are represented by the curves (Matsushima et al., 1997).

Figure indicates a feasible structural model of nine-helical zein protein. Figure shows a feasible model for arranging zein proteins within a cell and stacking molecular structures.

Furthermore, using SAXS, Matsushima et al. (1997) proposed that the tandem antiparallel helices (each shaped by a separate α -cell and linked by glutamine-rich curves or loops) stack linearly in the orientation perpendicular to the helical curve. The proposed α -zein organizational system adopts an enlarged triangular panel form with 13 nm size and an estimated 6:1 rotational proportion. SAXS results also suggested that the reduced α -zein exist as an aggregate of four zein molecules rather than monomers in aqueous ethanol solution.

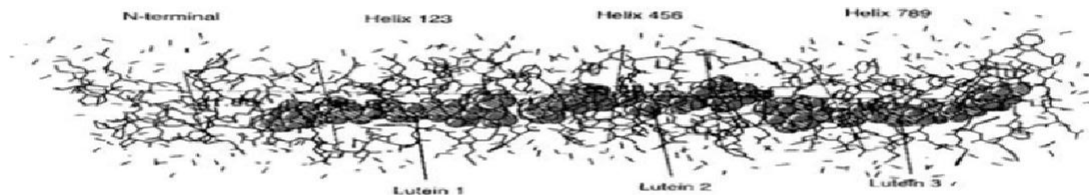


Fig-3 Complete structure of Z19 after coupling all triple-superhelix segments and adding the N-terminal segment (Momany et al., 2006).

More recently, Momany et al. (2006) said that the α -zein structures previously published do not agree with the experimental data and created a completely new model using simulations of molecular mechanics and dynamics. They proposed three superhelical (quad strand) constructions; each composed of three helical sections parallel with each other in an antiparallel path and linked by a bend or curve wealthy in glutamine.

Momany et al. (2006) discovered that this superhelical section can be constructed using only the coiled-coil conformations. All nonpolar images are on the inside of the triple-helix section, while the end strands of glutamine point back from the helical area.

Chapter - 4

RESULT AND DISCUSSIONS

4.0 Synthesis of Zein Film

1.5gm Zein powder was mixed in 15ml of 90% ethanol solution and was then stirred at 60C for 45min and while stirring Glycerol(120l) and Essential oil(120l) was added. Solvent was casted by Solvent Casting Tecchnique and then it was kept in oven at 25C for 24 hours and the required film was formed as shown in figure 3.



Figure 4: a) Zein Solution

b) Zein Film

4.1 FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The film was characterized with IR to know about the functional group present. Due to peptide groups the internal peaks are shown in the protein spectra. In this spectra proteins are characterized as amino group peaks, N-H₂ bending (1° amines) at 1468.2 cm⁻¹. In the spectrum, the peak at 2884.8 cm⁻¹ falls in the the broad band from of CooH groups. The bands in range 921.9 to 989.4 cm⁻¹ is of -CH₂. The peaks at 1468.2 cm⁻¹ for -OH and 1112.96 cm⁻¹ for C=O suggested the presence of alcohol or acid groups in zein films. Similar results have been reported by [Khalil *et al*, 2014]

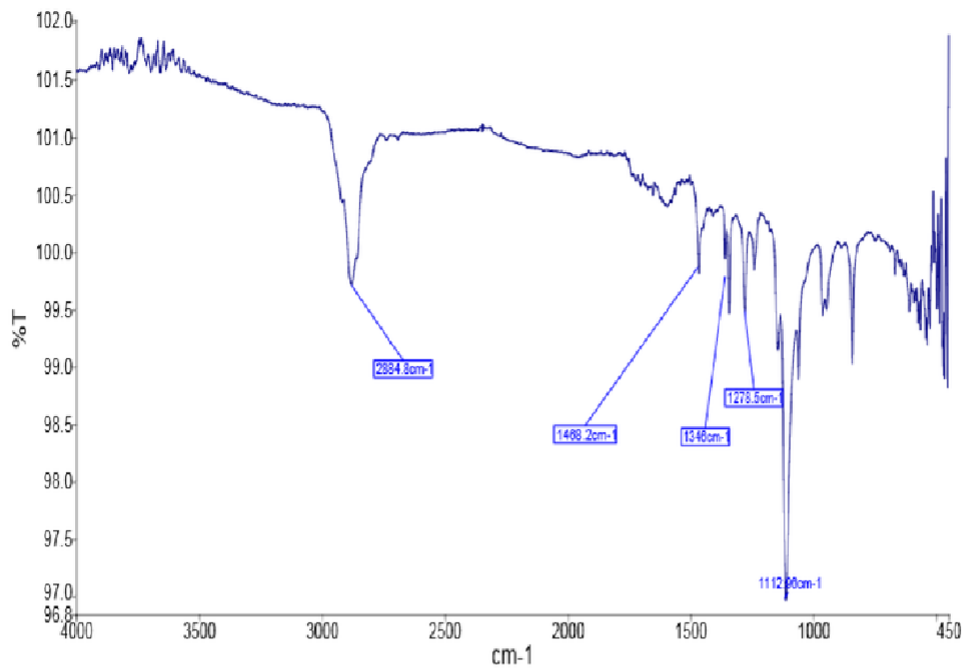


Fig:5 FTIR analysis of Zein film

4.2 Thermal Stability

The TGA(Thermogravimetric Analysis) was used to explore the thermal stability of Zein by evaluating mass loss induced as a result of temperature by volatile compound formation. The TGA graph as shown in Fig generally show two phases of inert thermal degradation. The first stage was the loss of low molecular mass compounds, i.e. from 70 to 200 C i.e plasticizers and solvent. Protein degradation was associated with the second stage (250–300 C). The rate at which the product begins to degrade is thought to be the most significant criterion for assessing polymer heat stability. Similar results have been reported by [Luecha *et al*,2010]

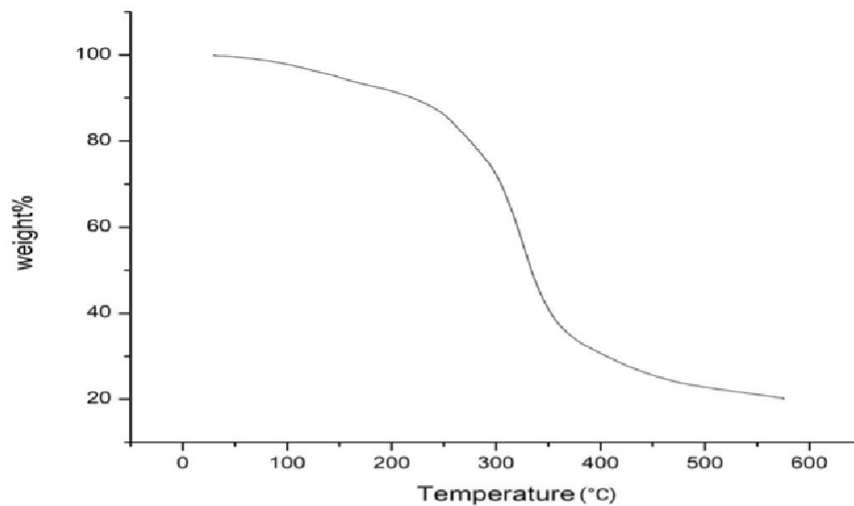


Fig-6: TGA Curve of Zein film

4.3 XRD (X-Ray Diffraction)

The XRD patterns of Zein film is shown in fig. The interlayer spacing has been calculated from the diffraction peak with the help of Bragg's equation ($n\lambda = 2d \sin \theta$). It can be noticed that the film has diffraction peak at 21.4° with the interlayer spacing of 18.27 \AA . Similar data has been reported in literature. This study suggests that there is intercalation in zein matrix which leads to crystallinity of the film.

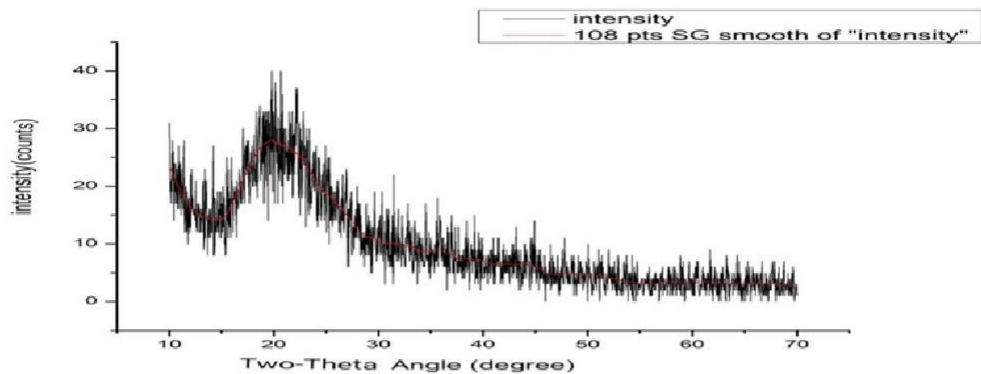
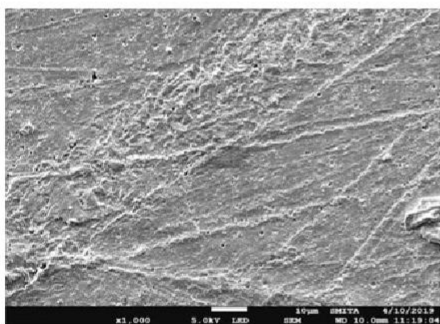


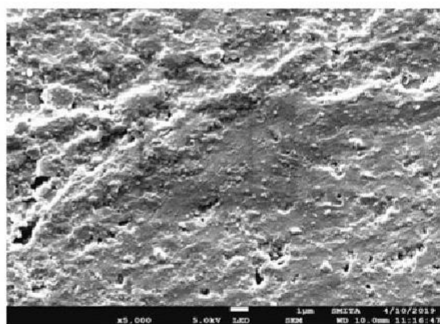
Figure 7: XRD Pattern of Zein film

4.4 SEM (Scanning Electron Microscope) Analysis

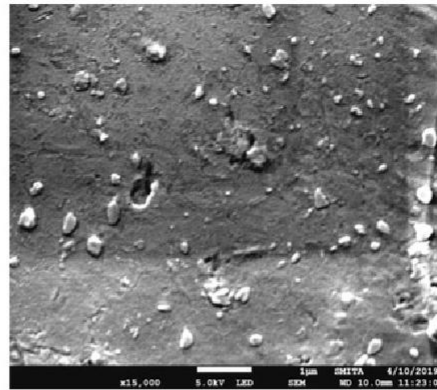
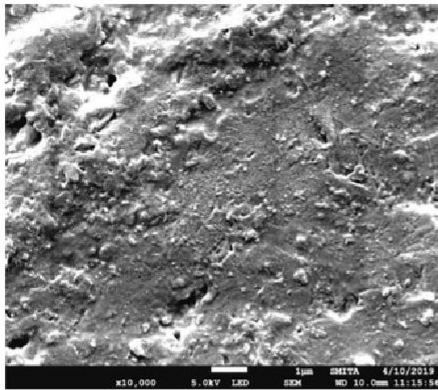
In this study, morphology of Zein film crosslinked with Glycerol and Essential oil is studied.



a) 1000X



(b) 5000X



(c) 10000X

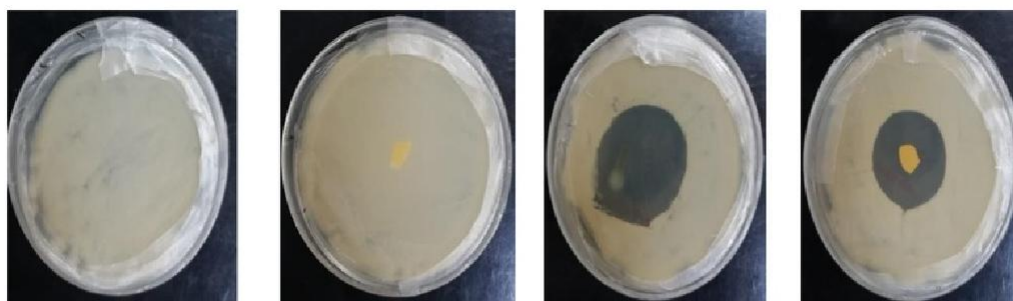
(d) 15000X

Figure 8: SEM Images of Zein Film

We can see some pores in the fig. In some cases pore formation in some polymer matrix can be due to presence of crosslinker[Khalil *et al*,2014].There is clearly rough surface ,discontinuous matrix in presence of crosslinker , these are in accordance with literature[Pereda *et al*]. Protein matrix and crosslinkers are the reason behind white and dark area in the micrographs of Zein films[Lai & Padua *et al*,1997].Some black points we can see are microbubbles which are entrapped in the polymer matrix or spaces which are taken by glycerol before the process of drying[Gounga *et al*,2007].

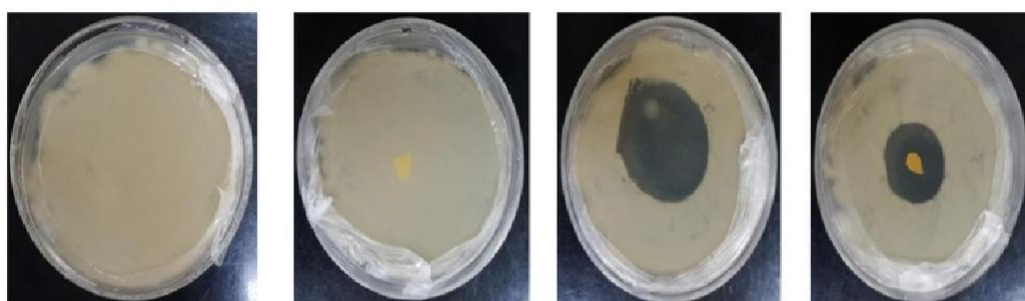
4.5 Antimicrobial Activity of Zein Films

Antimicrobial packaging is a successful type of effective food packaging as microbial contamination of food happens mainly on the surface. In order to explore the option of using zein-based film cross-linked with numerous cross-linking agents in active packaging, the antimicrobial impacts of the films acquired were evaluated against different pathogenic bacteria.



(a) S.Aureus (Blank) (b)S.Aureus with Zein (c)S.Aureus with EO (d)S.Aureus with ZEO

Figure 8 Antimicrobial results of Zein films with S.Aureus



(a)E.Coli (Blank) (b)E.coli with Zein (c)E.Coli with EO (d)E.Coli with ZEO

Figure 8 Antimicrobial results of Zein films with E.Coli

Zein film which have been crosslinked with Essential oil was effective and showed strong Zone of Inhibition against both *S.Aureus* and *E.Coli* while normal zein film without Eo didn't show any antimicrobial property against these bacterial species which is in accordance with literature[Khalil *et al*,2014]

4.6 Contact Angle Measurement

Contact Angle basically tells about the hydrophobic/hydrophilic character of the film surface. The angle that is formed between the water droplet and surface of the film is measured. If the surface is more hydrophobic more will be the contact angle.

Contact angle of zein film depends on concentration of zein ,type and concentration of plasticizer used.

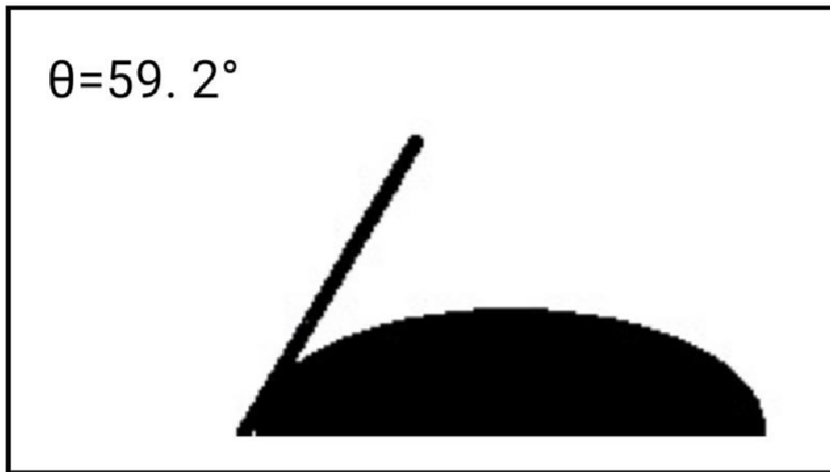


Figure 11 Contact Angle of Zein Film

Zein protein consists of numerous non polar amino acids and it is considered as a hydrophobic protein that's why a high value of contact angle in the range of 60-70 and it is in accordance with literature [Ghanbarzadeh *et al.*, 2006] [Muthuselvi *et al.*, 2006]. Addition of plasticizer leads to decrease in contact angle[Tihminlioglu *et al.*,2010].In our study, it was found to be 59 degrees.

4.7 Oxygen Permeability

Shelf life of food products is enhanced when oxygen barrier property of the film is high.The oxygen Transmission Rate of Zein film has been measured at 23C & 0% RH(Relative Humidity).If higher amount of zein has been present in formulations then it has given lower value of oxygen transmission rate[Cuq *et al.*,1998],[Rakotonirainy *et al.*,2001] .Zein has lower value of oxygen permeability than LDPE(Lower Density Polyethylene),HDPE(High Density

Polyethylene),PS(Polystyrene),PVC(Poly Vinyl Chloride)[Shin & Coworker *et al*,2002].In our study we have oTR value of Zein film with Eo comes out to be (0.0705 cc/m² ×day)which is much better than oTR value of corn-zein film(44.0 cc/m² ×day) in accordance with literarture[Tihminlioglu *et al*,2010] having GLY/50(Plasticiser type/Concentration)& 95/5(Ethanol%(v/v)/Corn-Zein(w/v) concentration.

4.8 Water Vapour Permeability

In accordance with the ASTM E96 normal technique, water vapor permeability (WVP) was determined. Water Vapour Permeability is determined after testing the WVTR of sample placed under particular temperature(25C) and relative humidity(60%).The value of WVTR is calculated as follows $WVTR=G/tA$

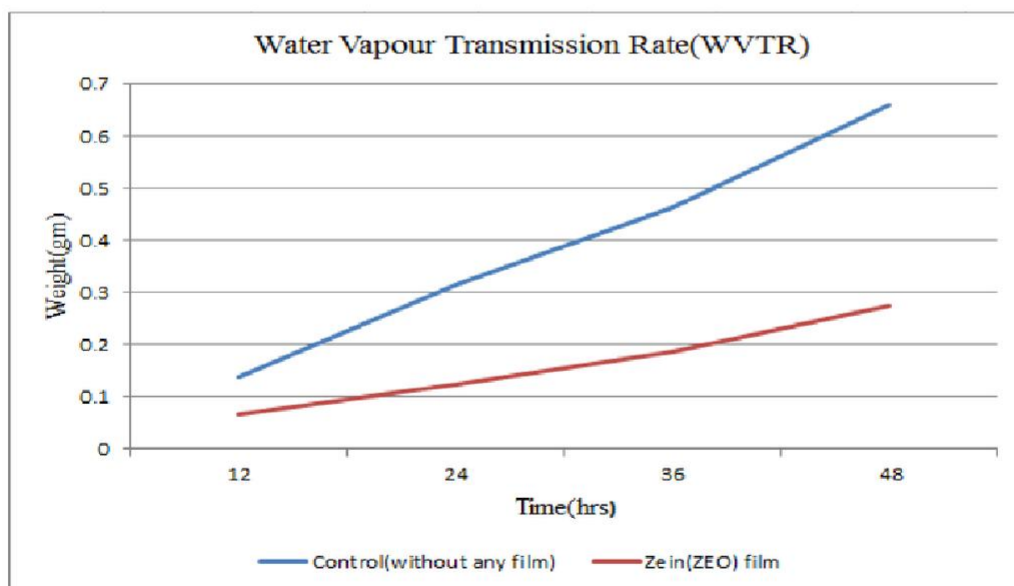


Figure 12. Water Vapour Transmission Rate Graph

Films with higher quantities of zein have higher hydrophobicity thus, increasing the water barrier properties.The value of WVTR of zein film so obtained was 0.0011685×10^4 gm/hr.m².

4.9 Tensile Testing

The mechanical properties of the film is tested by Instron Micro Tensile Tester Model 5848, Singapore .With film dimension(1cm*5cm), Load cell of 10N ,Speed of Elongation (5mm/min) the mechanical properties of the film(Thickness: 0.295mm) has been evaluated.

To accommodate stress/load from outside environment packaging film need to have good mechanical strength and extensibility. Zein film which have been prepared without any plasticiser were brittle[Yang & Coworkers,1996]. Zein films which are prepared using ethanol(88%) gives tensile strength of 0.99Mpa. When cross-linking agents are added to pure zein film higher value of tensile strength were obtained almost two- to three folds[Khalil *et al*,2014]Cross-linking molecules penetrate into the polymer network of Zein might be the reason which has lead to structural changes and hence the mechanical property. In this study Tensile Strength of the Zein(ZEo) film is found to be 46N psi.

Conclusion

The protein films have benefits over synthetic movies, including their use as organic packaging products. Moreover, opposed to those made from lipids and polysaccharides, these films have remarkable gas barrier characteristics. Because proteins have a unique structure, the mechanical properties of protein-based edible films are also better than those of polysaccharide and fat-based films. Technically, protein-based films characteristics rely on the protein sort, polymer chemistry, handling environments, and additives used.

Edible protein-based films combined with hydrophobic fabrics and synthetic polymer can lead to stronger performance than protein-only films, particularly with regard to their mechanical and barrier characteristics. Irradiation has been discovered to be an efficient technique for improving the barriers and mechanical characteristics of culinary protein-based films. Protein-based culinary films demonstrate remarkable gas barrier; therefore, protein films would be an appropriate food packaging product to prevent the development of aerobic microbial and lipid oxidation in products enhanced with lipids.

Fabrication and Characterization of Zein Film for Sustainable Food Packaging

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