Process development of biodegradable chitosan-based films and their suitability for food packaging

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in

BIOTECHNOLOGY

By

P.C. Srinivasa, B.E., M.Tech.

Department of Biochemistry and Nutrition
Central Food Technological Research Institute
Mysore-570020, India

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Dr. R.N. Tharanathan
Deputy Director

Biochemistry and Nutrition
Date: 21-12-2004

CERTIFICATE

I hereby certify that the thesis entitled "**Process development of biodegradable chitosan-based films and their suitability for food packaging"** submitted by Mr. P.C. Srinivasa for the award of the degree of ***Doctor of Philosophy in Biotechnology*** to the University of Mysore, India is the result of the research work carried out by him in the Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, Under my guidance during the period 1999 - 2004.

Dr R.N. Tharanathan
(Guide)

DECLARATION

I hereby declare that the thesis entitled, "**Process development of biodegradable chitosan-based films and their suitability for food packaging**" submitted by me to the University of Mysore, India for the award of the Degree of Doctor of Philosophy in Biotechnology, is the result of the research work carried out by me in the Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, under the Guidance of **Dr. R.N. Tharanathan** during the period 1999– 2004.

I further declare that the results presented in this thesis have not been submitted for the award of any other degree or fellowship.

Place: Mysore **(P.C. SRINIVASA)**
Date : 21-12-2004

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ABBREVIATIONS

AACC	American Association of Cereal Chemists
ASTM	American society for testing and materials
E	Average percent error
BET	Brunauer-Emmet- Teller
cm	Centimeter
Da	Dalton
B	Degree brix
C	Degree centigrade
DD	Degree of deacetylation
DSC	Differential scanning calorimetry
X_e	Equilibrium moisture content
ERH	Equilibrium relative humidity
X_f	Final moisture content
FTIR	Fourier transform infrared spectroscopy
GPC	Gel permeation chromatohgraphy
g	Gram
GAB	Guggenheim-Anderson-de Boer
hr	Hours
X_o	Initial moisture content
η	Intrinsic viscosity
kPa	Kilo Pascal
LDPE	Low density polyethylene
MPa	Mega Pascal
mPas	Milli Pascal
mg	Milligram
ml	Milliliter
min	Minutes
ME	Modulus of elasticity
X_{db}	Moisture content at any time

MR	Moisture ratio
M_w	Molecular weight
OTR	Oxygen transmission rate
ppm	Parts per million
PHA	Pollyhydroxy alkonates
PEG	Polyethylene glycol
PVA	Polyvinyl alcohol
PCA	Principal component analysis
RH	Relative humidity
RMSE	Root mean square error
SEM	Scanning electron microscopy
S_D	Standard deviation
TS	Tensile strength
a_w	Water activity
WRV	Water retention value
WVP	Water vapour permeability

Contents

Plastics have become part and parcel of our everyday life and the plastic industry has emerged as a rapidly expanding industry in the past several decades. Approximately 40,000,000 tones of plastic packaging is used annually world wide, and a majority of this is put to one time use and is discarded later. This contributes to an appreciable amount of total waste stream (around 20% volume world wide) and in India its contribution is approximately 3 million tones. The treatment of waste plastics has become a serious problem because of the difficulty of ensuring reclaimed land and burning by incineration. The industry is now facing ecological and legislative issues for handling plastic raw materials and finished products. Their total non-biodegradability as well as an increased environmental consciousness by the consumers and Government bodies has paved the way to look for alternate approaches. Also due care is necessary not to deteriorate the environment by using non-biodegradable and non-recyclable materials. This development has for the best part led to focusing on alternative packaging films derived from natural biopolymers which are replenishable and completely biodegradable under a variety of ecological systems. Biopolymer films are generally prepared by using biological materials such as polysaccharides, proteins and their derivatives, which are naturally and abundantly available. Natural biopolymeric films have the advantage over synthetic biopolymers since they are totally biodegradable and are derived from renewable raw materials. They can be used effectively as an alternative to synthetic plastics. Biopolymeric films have also desirable overall mechanical and barrier properties.

Food packaging is an important discipline of food technology concerned with the protection and preservation of all types of foods from oxidative and microbial spoilage. The petroleum based synthetic thermoplastic materials, currently being used extensively, may gradually loose importance as packaging materials because of waste disposal and non-biodegradable problems, and as a consequence threat to the environment. As an alternative, interest in the study of biodegradable packaging films has

increased steadily during the past decade. Although it is not feasible to entirely replace synthetic plastic packaging films, the biodegradable films do have potential to reduce and replace plastic packaging films in some specific applications. A clean pollution free environment is the need of the day.

Polysaccharides such as cellulose and starch (derived from agricultural resources), chitin/chitosan (derived from marine food processing wastes) and pullulan (from microbial sources), either in their native or modified forms, as well as their blends have the ability to form films. Bioplastic consisting of synthetic monomeric or polymeric materials, graft copolymerized with natural biomolecule are also shown to be useful as biodegradable packaging materials. Use of certain additives such as plasticizers, antioxidants and antimicrobials will enhance their functional value to a great extent. Use of chitosan and its derivatives in such applications has the additional advantages of being biocompatible and antimicrobial.

Chitin, a naturally occurring and abundantly available polysaccharide obtained form crustacean wastes, consists mainly of β (1-4)-linked-2-acetamido-2-deoxy-D-glucose units. Chitosan is obtained from chitin by N-deacetylation using strong alkali. The cationic property of chitosan offers an opportunity to take advantage of its electrostatic interaction properties. Chitosan films are used in the separation of ethanol from water by evaporation, water purification, and controlled release of pharmaceuticals, but has been reported to have limited application as far as packaging film is concerned. Therefore, it was felt desirable that a study be initiated to evaluate the properties of chitosan film prepared under different drying conditions and to modify the films by incorporating various additives and to look for their application to storage studies of fruits, vegetables, dairy and bakery products, and also to study their antimicrobial properties. With these objectives in mind, work was carried out and the results obtained are consolidated in the form of a thesis having the following layout.

Chapter 1

This chapter provides a General introduction of the subject matter, with reference to problems due to non-biodegradable plastics, different ways of handling these plastics and different sources of biodegradable plastics. The main focus is on occurrence and distribution of chitin/chitosan, their chemical structure, physicochemical properties, and application in various fields including food, medicine, agriculture and industry. Emphasis is given on the preparation of chitosan-based films and their application to shelflife extension of fruits, vegetables and other products. Finally, the Aim and Scope of the present investigation are indicated.

Chapter 2

This section includes a brief introduction on the preparation of chitosan films, with a detailed account of characterization of chitosan samples from two different sources. Chitosan sample (CH1) is of molecular weight 1,00,000 Da with Degree of deacetylation (DD) of 83%, whereas chitosan sample (CH2) is of molecular weight 2,00,000 Da with DD >90%. Chitosan films were wet casted on different base materials such as glass, Teflon, aluminium sheet, stainless steel sheet and polyester sheet for easy peeling off from the base, of all the polyester base gave the best quality of film. Chitosan films were prepared by using different (solvent) acids such as acetic acid, lactic acid, formic acid and propionic acid and their properties studied. Formic acid cast film had a higher tensile strength (48.34 ± 4.28 MPa) and lactic acid cast films had the lowest value (21.9 ± 4.2 MPa). On the other hand, chitosan films prepared using acetic acid showed easy handling and good film properties. Chitosan films were prepared under different drying conditions, such as ambient drying, oven drying and infrared drying. The results showed IR drying to be faster and superior in preserving desirable functional characteristics of chitosan films. Though subtle variations in the crystallinity pattern were observed between differently dried

chitosan films, no significant differences were observed in their mechanical and barrier properties. Typical drying curves were been obtained for films dried at different drying conditions, and their kinetic constants determined. The sorption curves of the films showed a sigmoid shape and WVTR of the films showed an increase with higher relative humidity. The tensile strength (TS), percent elongation (%ε) and modulus of elasticity (ME) of films were studied at different RH, temperature, and storage period using response surface methodology. Simultaneous optimization by desirability approach resulted in an overall desirability score of 0.8429, where in TS, %ε and ME values were 35.79 MPa, 19.86% and 896.73 MPa and these values were obtained when the independent variables such as temperature, RH, and storage days were 20.1°C, 40% and ~7 days, respectively. Lastly an attempt was made to fabricate a prototype model for continuous preparation of chitosan film under infrared drying condition.

Chapter 3

This chapter describes blending of chitosan with polyols (glycerol, sorbitol and PEG), fatty acids (stearic acid and palmitic acid) and a water-soluble polymer, PVA, before casting the films. The optical properties (colour density), mechanical properties (tensile strength, %ε, ME, tearing strength, burst strength and impact strength), barrier properties (WVP and OTR) were all determined. The chemical nature of these films was studied by FTIR, heat flow by DSC and changes in crystallinity by X-ray diffractogram. The sorption isotherms of all blend films were studied and observed for validity of different models.

The result indicated that yellowness of the film increased with the addition of plasticizers. The opacity of film increased with the addition of PEG but no differences seen in glycerol and sorbitol added films. TS decreased to 6.08 and 6.24 MPa in glycerol and sorbitol containing films respectively, whereas in PEG both decreasing and increasing trends were

noticed. The ME also showed a decreasing trend. The Impact strength was increased with the addition of polyols. Burst strength of the film decreased with addition of glycerol and sorbitol, but in PEG an increasing trend with a value of (190 kPa) was observed. The WVP of native chitosan film was 0.01322 g.m/m^2.day.kPa. With the addition of glycerol the WVP decreased to 0.008 and in sorbitol it increased to 0.0163 g.m/m^2.day.kPa. In PEG films it was 0.019 g.m/m^2.day.kPa. The OTR values decreased with the addition of PEG, whereas with glycerol and sorbitol it increased to 98.01 and 141.14 x 10^{-6} cc.m/m^2. day. kPa, respectively.

DSC thermogram showed a difference in the ΔH values for various polyol containing films, ΔH values increased as glycerol concentration increased in the blend films. Water capacities of the blend films showed different characteristics, glycerol showed a early evaporation at around 125 °C with high ΔH values, compared to other two plasticizers. X-ray diffraction of polyol blend films showed no significant differences.

With the addition of fatty acid the density of blend film decreased from 1.4024 to 1.2692 g/ml in palmitic acid and 1.4024 to 1.2585 g/ml in stearic acid blend film. TS of blend film decreased with the addition of fatty acid and no significant variation was observed in % elongation and modulus of elasticity. The WVP results showed no significant differences. The FTIR showed hydrogen bonds between hydroxyl groups and water molecules to remain intact. The methyl and methylene stretching appeared at around 2918 and 2850 cm^{-1}, which were attributed to amide stretching. The palmitic and stearic acid blend chitosan films showed melting peaks at around 63 °C and 56 °C, respectively, and noticeable water content were found in these films.

The optical properties and TS of the chitosan-PVA blend films increased while %elongation decreased with increase in chitosan concentration and a blend ratio of 60-40 was found to be the best which had

a value of 41.24 MPa. The burst and impact strength of the blend films increased with the addition of PVA. The increase in the impact strength was attributed to the chain flexibilities of the blend films. The WVP of films decreased to 0.006 g.m/m^2.day.kPa. FTIR spectrum showed a characteristic peak shifting to a lower frequency range due to hydrogen bonding between -OH of PVA and –OH or NH_2 of chitosan. The blending ratio showed a regression coefficient of 0.94. DSC thermogram of chitosan-PVA showed endotherm around 140-160° C and 215° C. The exotherm peak of chitosan at around 300° C was diminished as PVA concentration increased due to overlapping of PVA endotherm. X-ray diffraction patterns showed 2θ peaks 11.92°, 21.28° and 23.28°, the latter was due to drying of chitosan acetate salts. The intensity of peak around 19° increased as the concentration of PVA increased. Moisture sorption isotherms showed sigmoid pattern, indicating the influence of polyols/fatty acid/PVA on the blend film. Sorption data were useful in choosing suitable packaging material having a desirable water vapour barrier property. The GAB model showed a better fit compared to other models and was applicable to a wide range of water activity values. Chitosan blend films with polyols and fatty acids showed complete bio-degradation.

Chapter 4

This chapter describes storage studies of fruits and vegetables, dairy and bakery products using chitosan film. Shelflife extension of mango fruits was studied and compared with the sensory profile. Control fruits were found spoiled in 10 days, whereas chitosan covered fruits showed better sensory quality. The latter were observed with high levels of carotenoids, sugars, free from off-flavour and fungal growth compared to LDPE covered fruits. In PCA plot the chitosan-covered fruits showed several desirable quality attributes. At the end of storage period (20 days), the chitosan covered fruits showed better sensory quality than LDPE covered fruits.

Tomatoes stored in chitosan covered cartons showed uniform colour development, free from off –flavour and retention of sugar level for more than 30 days of storage, compared to control fruits which had 15 days of storage, while LDPE packed tomatoes showed non-uniform colour development. Similarly, chitosan and LDPE packed bell pepper pods showed a shelflife of 16 days. The textural studies data showed that modified atmosphere packaged conditions can extend the shelflife of tomato and bell pepper, which are beneficial for sustainable fluctuating market availability associated with limited and seasonal availability. No differences were observed in headspace gas levels during the storage period, while greater changes were observed in colour development and its retention. Changes in chemical parameters of stored fruits were very marginal. Sensory profiling indicated that synthetic film packaged fruits exhibited loss of typical aroma, while chitosan packaged fruits retained it. Firmness and development of red colour and retention of green colour are the major factors in deciding the price and market value of tomatoes and bell pepper, respectively. The unpackaged fruits showed decaying symptoms at an early stage than the packaged samples, which indicated a beneficial role of chitosan films for extending the shelflife of bell pepper.

Dairy product (peda) stored in chitosan coated butter paper gave considerably extend storage. A similar trend was seen in the package of bakery product (bread). Further, incorporation of chitosan into the dough gave an additional 30 days of shelflife extension.

Chapter 5

This chapter deals with antimicrobial characteristics of chitosan film. Our results conclusively demonstrated the antimicrobial efficiency of chitosan film even at very low concentrations in liquid medium. Although there was lesser diffusion of chitosan on the agar surface, there was no

visible microbial growth, which makes it a potential packaging film for use in food preservation. Growth curve of microorganisms showed effectiveness of chitosan film in inhibiting growth of microbes. SEM studies revealed the effectiveness of chitosan film as an antimicrobial agent. A chitosan-based film with a broad spectrum of antimicrobial activity will have a higher potential as food packaging material.

The presentation is finally concluded with a note on the Highlights of Significant Findings from this investigation, followed by Bibliographic listing of the literature referred to in preparing this thesis.

P.C.SRINIVASA
(Candidate)

R.N.THARANATHAN
(Guide)

Environmental deterioration is directly the outcome of pollution of soil, air and water. The spread of industrialization and agriculture has been considerably responsible for highly toxic chemicals entering into the natural streams through industrial and municipal effluents. Prevention and control of pollution is therefore, a necessity. It is vital to bring about reconciliation between development and conservation of environment and ecology. The concept that food supply must nutritionally be adequate, equitably shared, socially affordable and predictable leads to intensive agricultural practices for enhanced food production, better storage and preservation, and diversified processing and packing methods. The packaging requirements including foodstuffs are far more diverse and complex than those of other non-food products. The primary functions of a packaging material are to protect, present, and dispense the products.

Packaging is important in post harvest preservation of fruits and vegetables and processed foods for assured shelflife extension. The raw materials of food as well as the innumerable number of processed food products made available in the market are highly perishable and need effective and efficient packaging systems for extending their shelflife and availability, especially at far off places (Kittur *et al.*, 1998). Protection of food products relates to the rate of quality change, including both physical (mechanical damage during transit or storage, loss of consistency or crispiness, loss of appearance, and sales appeal) and organoleptic changes (loss of taste, colour and odor) (Ashley, 1994). Thus, the package should protect the food product against physical hazards and atmospheric/ environmental factors such as water vapor, gases and odors. By a suitable combination of structural design and material selection, food packaging must ensure a condusive environment inside and preserve the food products by maintaining the internal gaseous atmosphere and also be effective against external deteriorative influences. A number of different synthetic polymers, in combination, which are often petroleum based, are employed when their individual properties are of benefit in providing the required protection.

Usually synthetic polymer packaging materials may be combined together by processing such as lamination, extrusion coating or co-extrusion to form multilayer structures, which can be subsequently formed into flexible pouches, wraps, tubes, or containers (Hong and Krochta, 2003).

Regarding the barrier properties, the critical compounds that can penetrate (both from outside-in and inside-out) the packaging materials and degrade food quality are the water vapor and oxygen (of the surrounding atmosphere). While water is only weakly and reversibly held to foods by hydrogen bonding, oxygen is strongly and irreversibly reacted. The ingress of oxygen leads to a permanent change (oxidative spoilage) in the nature of food products. Thus, protection from oxidative spoilage is one of the most important challenges in packaging of food products. Optimum oxygen barrier properties are crucial for achieving a long shelflife (Hong and Krochta, 2003).

Petroleum based plastics such as polyethylene, polypropylene, etc., have replaced traditional packaging materials such as metallic cans, glass containers, paper boards, bamboo, dry leaves of some perennial trees, etc. The first semi synthetic plastic, "celluloid" was prepared by reacting cellulose and camphor. Around the turn-off the century, researchers from Germany and France discovered casein- plastic material made by treating milk protein with formaldehyde, but it was not known until 1909 when Leo Baekeland created Bakelite, an entirely synthetic plastic based on phenol-formaldehyde resin. Subsequently, various other polymeric materials such as polyvinyl chloride, polyamide, polystyrene and several elastomers (synthetic rubber) were made use of to prepare synthetic packaging materials, with diversified properties and application potentials.

Presently we are experiencing POLYMER AGE era because polymeric materials have been replacing many of our day-to-day articles and have become an integral part of our present day life style. Starting from toothbrush to space shuttle, today we can't imagine life without the use of

plastic materials. Plastics are materials that can be shaped into virtually any form. Today almost all the available plastics are manufactured synthetically. The basic raw materials for all the modern plastics are crude oil and natural gas, which are mixtures of heavy hydrocarbons, processed in the petroleum refineries by fractional distillation to obtain Naphtha. Naphtha upon further cracking gives rise to lighter hydrocarbons, which are the raw materials for most of plastics used today and ethylene gas obtained during cracking is yet another raw material used for the preparation of commonly used plastics such as polyethylene, polyvinyl chloride (PVC), etc.,

Plastics and pollution

Indiscriminate use of plastics in packaging industry and littering all around without proper disposal management has led to mounting solid wastes and causing severe environmental pollution. The production and consumption of plastics have increased in geometrical progression. The annual per capita consumption of plastics in India is 2 kg/person/year compared to 60 kg/person/year in developed countries. In India, plastic wastes accounts to 3 percent by weight of total of 80,000 metric tones of municipal solid waste generated daily (Kalia *et al.,* 2000). In USA, out of 4 lakh tones of garbage generated daily, plastics constitute 30 percent of its volume and their disposal is causing new challenges.

The major hurdle against increased use of plastics is their total non-biodegradability and as a result, ever increasing–mounting garbage wastes. With the ever-growing environmental consciousness among consumers and by governments, the plastic packaging industry is now facing severe ecological and legislative issues for handling raw materials and eventual disposal of solid plastic wastes and finished products. Traditional methods for handling post consumer plastic wastes include incineration, depolymerization, recycling and land filling (Mody, 2000).

Incineration

Plastic wastes can be utilized as a renewable source of energy. Burning the plastic in a conventional incinerator involves high flame temperature, releasing a large amount of heat. PVC, constituting 3-4% of the refuse, when incinerated, gives out hydrogen chloride and free chlorine gas, which may react with unburnt hydrocarbons to form dioxin, a toxic pollutant. The CO_2 generated adds to the problem of green house effect. Other pollutants such as NO, SO_2, NH_3, etc. discharged into the environment also cause serious problems, particularly they cause health hazards, like lung cancer, skin diseases, asthma, etc.

Depolymerization

Olefinic plastics such as polyethylene (PE), polypropylene (PP), etc. are known to undergo photolytic, oxidative, thermal and catalytic chain scission. Thus, such plastic wastes can be partially depolymerized to monomers that can be reused. Nevertheless, the extremely severe conditions of temperature and pressure required for complete depolymerization make this route uneconomical (Mody, 2000).

Recycling

Thermo-softening plastics (PE, PP, PVC, etc) can be softened and remolded /recycled by application of heat and pressure. Recycling of plastics can provide only a part time solution to long-term reduction of plastics. However, during recycling the material looses some of the properties like appearance, chemical resistance, reprocessibility and mechanical characteristics.

Landfill

In the absence of economically feasible disposal methods, land filling is the ultimate solution for majority of the plastics. Being non-biodegradable,

the waste plastics remain buried for several years, causing ecological pollution. Nevertheless, in recent years, there is a global awareness about the need to reduce the amount of plastic wastes discarded in land fill, and slowly legislation is being brought forward for discouraging or minimizing the use of plastics.

Biodegradable or Biobased polymers

Of late, there is a paradigm shift imposed by all to look for processes and packaging films made out of biobased polymers, which are biodegradable and therefore compatible with the environment. In a sense, biodegradability is not only a functional requirement but also an important environmental attribute. Thus, the concept of biodegradability enjoys both user-friendly and eco-friendly advantages, and the raw materials are generally derived from either replenishable agricultural feed stocks or marine food processing industry wastes and therefore capitalizes on natural resource conservation with an underpinning on environmentally friendly and safe atmosphere. An additional advantage of biodegradable packaging materials is that on biodegradation or disintegration and composting they may act as fertilizer and soil conditioner, facilitating better yield of the crops. Though somewhat expensive, biopackaging is tomorrow's need for packaging especially for a few value added food products (Tharanathan, 2003). Biopolymers from agricultural feed stocks and other resources have the ability upon suitable blending and/or processing to result in such packaging materials, whose functionality can be better expressed by using in combination with other ingredients such as plasticizers and additives. A few potential uses of such biopolymeric packaging materials are

1. Use and throw, disposable packaging materials,
2. Packaging routine consumer goods for day to day use,
3. Disposable personal care materials,
4. Lamination coatings, and
5. Bags for agricultural mulching.

There are two types of biomolecules viz. hydrocolloids and lipids, which are generally used in combination for the preparation of biodegradable packaging films or composites. Individually they lack structural integrity and characteristic functionality. Composite films are in fact a mixture of these and other ingredients in varying proportions, which determine their barrier and mechanical properties. Sometimes, a composite film formulation can be tailor made to suit to the needs of specific commodity or farm produce. Phase separation encountered during the preparation of composite formulations is overcome by using emulsifying agents. Use of plasticizers such as glycerol, PEG, sorbitol, etc. in the film formulations or composites is advantageous to impart pliability and flexibility, which will improve handling. (Garcia *et al.,* 2000). Use of plasticizers reduces the brittleness of the film by interfering with hydrogen bonding between the lipid and the hydrocolloid molecule.

Biopolymer films cannot generally be extruded like synthetic plastic films, as they do not have defined melting points and undergo decomposition upon heating. Film formation generally involves inter- and intra-molecular associations or cross-linking of polymer chains forming a semi rigid 3D network that entraps and immobilizes the solvent. The degree of cohesion depends on polymer structure, solvent used, temperature and the presence of other molecules such as plasticizers.

The various naturally occurring biopolymeric materials of use in composite film making formulations are broadly classified into three main categories based on their origin and production,

Category 1. Polymers directly extracted or recovered from biomass-polysaccharides such as starch, cellulose and proteins (casein and gluten).

Category 2. Polymers produced by classical chemical synthesis using renewable biobased monomers, for example poly-lactic acid, a bio-polyester polymerized from lactic acid.

Category 3. Polymers produced by microorganisms, for example, pullulan produced by *Pullularia pullulans*

The three categories of biobased polymers are represented in Fig 1.1

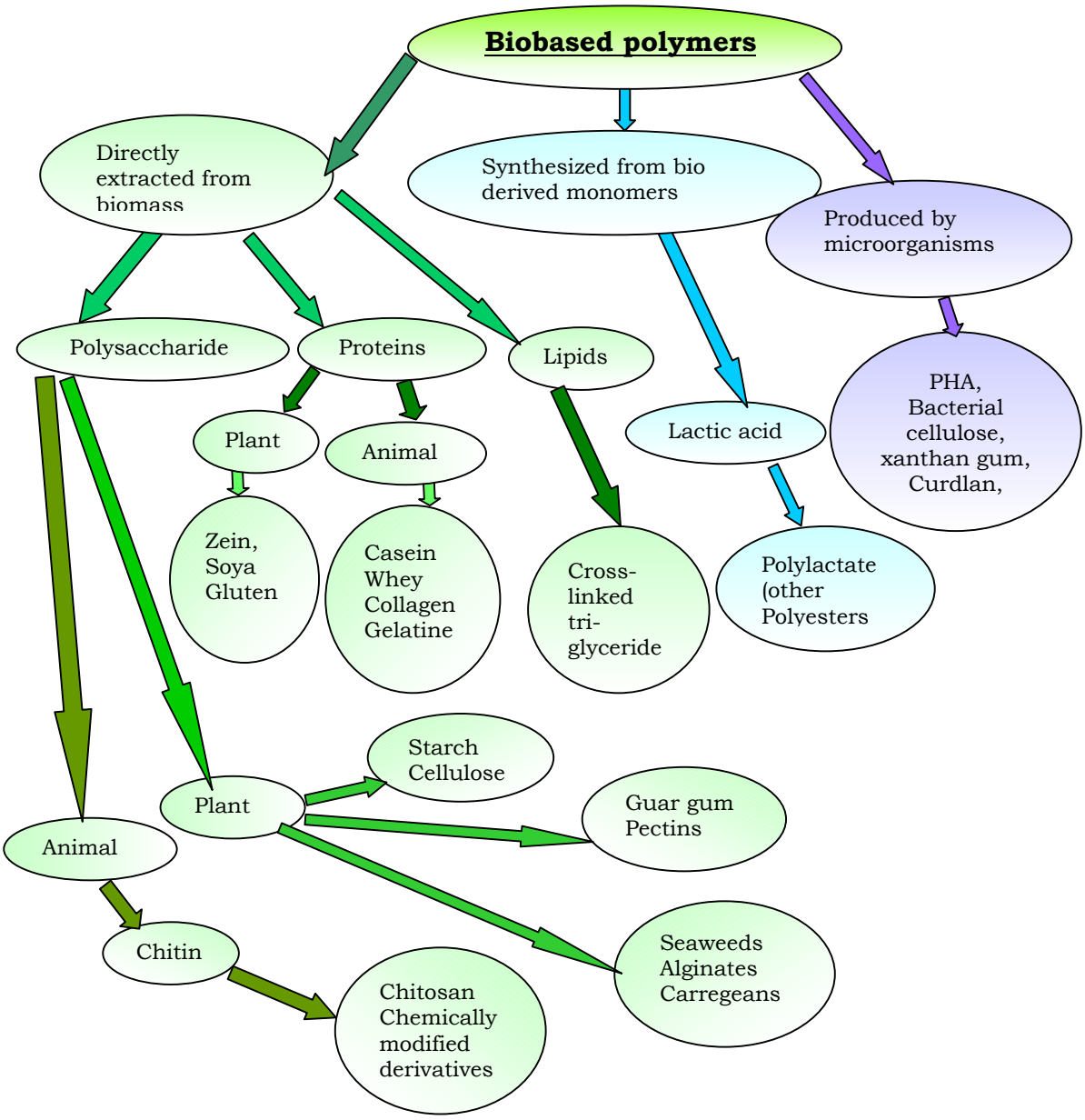


Fig.1.1. Different types of biobased polymers

The principal polysaccharides having the ability to form thin films are starch, cellulose, pectins chitosan and their derivatives.

(A). Hydrocolloids of plant origin- Polysaccharides -

Starch

Research on starch-based biodegradable plastics began in the 1970s and continues to date at various labs all over the world. Starch, is a storage polysaccharide of cereals, legumes and tubers, widely available, especially from corn, and having thermoplastic properties upon disruption of its molecular structure. (Tharanathan, 1995; Tharanathan and Saroja, 2001). Starch contains amylose and amylopectin, the former, especially in amylomaize starches containing 70% amylose gives stronger, more flexible films. Branched structure of amylopectin generally leads to films with poor mechanical properties (Tharanathan, 2003). As a packaging material, starch alone does not form good films with adequate mechanical properties, unless it is treated with plasticizers or blended with other materials. To overcome brittleness of starch-based films, common plasticizers like glycerol, sorbitol, etc. are used. Starch–based thermoplastic packaging materials are in commercial usage since last few years and are also dominating the market of biobased, compostable materials.

Cellulose and their derivatives

Cellulose is the most abundantly occurring natural polymer on earth. It is a linear polymer of anhydroglucose residues joined together by β 1,4-linkages. Because of its regular structure and array of hydroxyl groups, it tends to form strongly hydrogen bonded crystalline microfibrils and fibers. In the packaging context, the most familiar forms of cellulose are as paper or cardboard. A number of cellulose derivatives such as carboxymethyl cellulose, methylcellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose and cellulose acetate are used in the preparation of cellulose-based films. Cellulose acetate films are widely used in food packaging, since it has low gas and moisture barrier properties, which are in

a way dependent on the molecular weight of cellulose, the higher the molecular weight better is the properties (Krochta *et al.,* 1994).

Pectins

Pectins are a complex group of structural polysaccharides, which occur widely in land plants. The major commercial sources of pectins are citrus peel and apple pomace. Pectic substances are polymers mainly composed of (1->4)-∝-D-galactopyranosyluronic acid units. The solubility and gelation properties of pectins are differentiated by the degree of esterification (DE), which classifies commercial pectins into high-methoxyl (>50%) and low-methoxyl (<50%) pectins. The use of low-methoxyl pectinate as a coating agent in certain foods has been proposed, wherein it also gives an attractive, non-sticky surface to covered foods. These coatings have high water vapor permeability and the only way they could prevent dehydration is by adding other agents (Krochta *et al.,* 1994).

(B). Proteins

Proteins are of plant (gluten, soy, pea) or animal origin (casein, whey, collagen, keratin). Proteins are random copolymers of amino acid residues and their side chains are highly suitable for chemical modification for tailoring to the required properties of a packaging material (Tuil *et al.,* 2000).

Casein

Casein, a milk derived protein, can easily be processed due to its coiled structure. Upon processing with suitable plasticizers at temperatures of 80-100° C, film like materials can be made with mechanical properties varying from stiff, brittle to flexible and tough films. Casein melts are highly stretchable making them suitable for film blowing. In general casein films have an opaque appearance. The main drawback of casein films is their high

cost. It is generally used for bottle labeling because of its excellent adhesive properties.

Gluten

Gluten is the main storage protein of wheat and corn. During processing gluten leads to disulfide bridge formation formed by amino acid cysteine, which is relatively abundant in gluten. Plasticized gluten films exhibit high gloss and show good resistance to water under certain conditions. Its abundance and low cost are other factors encouraging continued research on the use of gluten films for various packaging applications.

Soy protein

Soy proteins are commercially available as soy flour, soy concentrate and soy isolate, all differing in protein content. Soy protein consists of two major fractions referred to as 7S (conglycinin, 35%) and 11S (glycinin, 52%) fractions, both containing cysteine residues, which lead to disulphide bridge formation during processing into films. (Fossen and Mulder, 1998)

Keratin

Keratin is by far the cheapest protein, extracted from waste streams such as hair, nail and feathers. Due to its structure and a high content of cysteine groups, keratin is the most difficult protein to process. After processing, a fully biodegradable, water-insoluble film is obtained, whose mechanical properties are very poor compared to other protein films.

Collagen

Collagen is a fibrous, structural protein of animal tissues, particularly bones, skin and tendons, having a common repeating unit. It is a flexible polymer, with a complex helical and fibrous structure. Collagen is insoluble

and difficult to process. Gelatin, prepared from collagen by acid hydrolysis, has a potential to form thin films. Gelatin is extremely moisture sensitive and for food applications its chemical modification is necessary.

Whey

Whey proteins, a by-product from cheese production, are particularly rich in β-lactoglobulin. They are of relatively high nutritional value, available in large amounts worldwide and have been extensively investigated as edible coatings and films.

Zein

Zein comprises a group of alcohol-soluble protein found in corn endosperm. Commercially zein is a byproduct of corn wet milling industry. Film forming properties of zein have been recognized for decades and film may be formed by solution casting or by extrusion technique (Ha, 1999; Lai and Padua, 1997). Zein films are brittle and need plasticizers to make them flexible.

(C). Polymers produced from polylactic acid

Polylactic acid (PLA), is a polymer prepared from lactic acid and has highest potential for a commercial major scale production of renewable packaging material. Lactic acid, the monomer may easily be produced by the fermentation of carbohydrate feed stock, such as maize, wheat, etc. PLA is chemically synthesized by condensation polymerization of the polylatate (Hakola, 1997). The properties of PLA are highly related to the ratio between the two mesoforms (L or D). L-PLA results in a material with very high melting point and high crystallinity. D-and L-PLAs are used as food packaging materials. PLA may be plasticized with its monomer or alternatively with oligomeric lactic acid. Being thermoplastic PLA may be formed into blown films and injection molded objects (Garde *et al.,* 2000).

(D). Polysaccharides produced by microorganisms

Polyhydroxy alkonoates (PHA) are structurally simple macromolecules synthesized by many Gram-negative bacteria. PHA's are reserve food materials under nutrient limited conditions. They are accumulated as discrete granules to level as high as 90% of the cells dry weight and are composed of 3-hydroxy fatty acids. In these polymers the carboxyl group of one monomer forms an ester bond with hydroxyl group of the neighboring monomer. Polyhydroxy butyrate – a class of PHA's has been studied more extensively and its presence has often been used as a taxonomic characteristic. PHA's are natural thermoplastic polyesters and hence majority of their applications are as replacement for synthetic plastics. PHA's can be processed into fibres, diapers, back sheets (Steel, 1996) and consumer packaging items such as bottles, pens, cosmetic containers etc. (Baptist, 1963).

Xanthan Gum

Xanthan gum is produced from the organism *Xanthomonas camqestris* by controlled fermentation. Each xanthan gum molecule contains five sugar residues, viz. two β-D-glucopyranosyl, two β-D-mannopyranosyl and one β-glucopyranosyluronic acid unit. Xanthan gum is readily dispersed in hot or cold water. It is used for thickening, suspending, and stabilizing effects in salad dressing, etc,. It can be used to provide uniform coating, good cling, and improve adhesion in wet batters, and to prevent moisture migration during frying.

Pullulan

Pullulan is a viscous polysaccharide extracellularly produced by fungus *Pullularia pullulans* or *Aureobasidium pullulans* commonly known as black yeast. Pullulan consists of maltotriose units polymerized in linear fashion with α-1-6-linkages with a degree of polymerization ranging from

100-5000. It is a whitish powder, non-hygroscopic and water-soluble. Pullulan films are transparent, colourless and oil and grease resistant. Pullulan can be used as coating formulation or packaging film material (Yuen, 1974).

(E). Polysaccharides of animal origin

Chitin/Chitosan

India is having 8129 kms of seashore and many varieties of fishes/ shellfish resources are available. The commercial catches of shrimp in India began in 1960's when trawling was introduced and seafood export became a major growing industry. In the year 2001-02 prawn products of 1,04,945 tons were exported. During crustacean processing, shell wastes accounting upto 60% of the original material are produced as a waste byproduct. One of the problems of seafood industries is disposal of this solid waste (Yogesh and Sachin, 2002). In 1970's, Environmental Protection Act (EPA) directed industries to stop dumping of shell wastes of crab, lobster and shrimp into sea/land. The shells are rich in $CaCO_3$, protein and a polysaccharide 'Chitin'. The name 'chitin' is derived from Greek word 'chiton', meaning a coat of mail or envelop. It is the second most abundant natural biopolymer after cellulose. Chitin is the major structural component of the exoskeleton of invertebrates, insects, yeast and cell wall of fungi. Chitin content in selected crustacean, insects, molluscan, and fungi is given in Table 1.1.

The major source of chitin is from invertebrates. Since biodegradation of chitin in crustacean shell waste is very slow, accumulation of large quantities of discards from the processing of crustaceans has become a major concern in the seafood industry in costal area (Shahidi and Synowiecki, 1991). The total global annual estimate is around 1600 million.

Table 1.1. Chitin content of selected crustacean, insects, molluscan organs and fungi

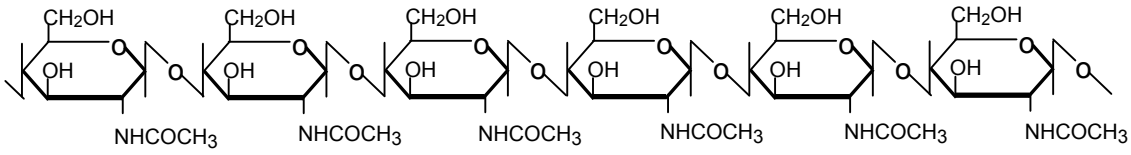
Type	Chitin content (%)	Type	Chitin content (%)
Crustacean		Pieris (sulfer butterfly)	64.0 [c]
Cancer (crab)	72.1 [c]	Bombyx (silk worm)	44.2 [c]
Carcinus (crab)	64.2 [b]	Calleria (wax worm)	33.7 [c]
Paralithodes (King crab)	35.0 [b]	**Molluscan organs**	
Callinectes (Blue crab)	14.0 [a]	Clamshell	6.1
Crangon (shrimp)	69.1 [c]	Oyster shell	3.6
Alasakan shrimp	28.0 [d]	Squid, Skeletal pen	41.0
Nephrops (Lobster)	69.1 [c]	Krill, deproteinized shell	40.2
Homarus (Lobster)	60-75 [c]	**Fungi**	
Lepas (barnacles)	58.3 [c]	*Aspergillus niger*	42.0 [e]
Insects		*Penicillium notatum*	18.5 [e]
Periplaneta (cockroach)	2.0 [d]	*Penicillium chrysogenum*	20.1 [e]
Blatella (cockroach)	18.4 [c]	*Saccharomyces cereviseae*	2.9 [e]
Colcoptera (beetle)	27-35 [c]	*Mucor rouxii*	44.5
Diptera (truefly)	54.8 [c]	*Lactarius vellereus* (mushroom)	19.0

a. Wet body weight, b. Dry body weight, c. Organic weight of cuticle
d. Total dry weight of cuticle, e. Dry weight of the cell wall.

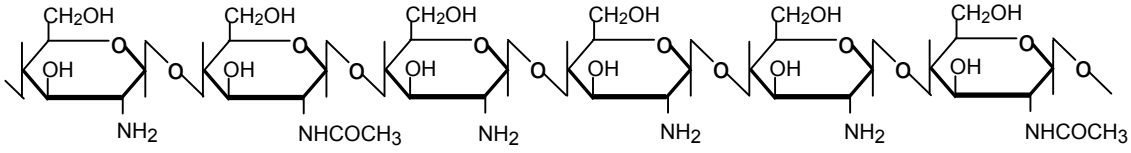
tons (Synowieecki and Al khateeb, 2000), Hence production of value-added products from such wastes and their application in different fields is of utmost interest. By a simple demineralization (by treatment with hot dil. HCl) and deproteinization (by treatment with hot dil. NaOH) steps, an amino polysaccharide 'Chitin' can be quantitatively recovered from crustacean wastes (Knorr, 1984). Chitin has been known to form microfibrillar

arrangement in living organism. The fibrils having a diameter of 2.5-2.8 nm are usually embedded in protein matrix, crustaceans cuticle possess chitin microfibril with diameter as large as 25 nm [Ravi Kumar, 2000).

Chitosan is the N-deacetylated derivative of chitin (by treatment with hot alkali), its structure is composed of 2-amino-2-deoxy-β-D-glucose (D-glucosamine) in a β(1-4) linkage, and with occasional N-acetyl glucose-amine residues. The structure of chitin and chitosan resembles cellulose except at position C-2, being replaced by acetamido and/or amino groups, respectively (Fig.1.2).



Chitin



Chitosan

Fig.1.2. Chemical structure of chitin and chitosan

Isolation of Chitin /Chitosan

The production of chitin and chitosan is currently based on crab and shrimp shells discarded by the sea food-canning industries. Since chitin is firmly associated with other constituents, harsh acidic/alkaline treatments are required to remove them from chitinous material (Fig.1.3), outlines some important steps in the extraction of chitin and chitosan. Initially proteins are removed by treating with hot sodium hydroxide solution. Minerals such as

calcium carbonate and calcium phosphate are extracted with hot HCl. Decolouration (bleaching) was done by treating with hydrogen peroxide or sodium hypochlorite solution. Chitin is washed and dried. To get chitosan, chitin was again treated with strong sodium hydroxide at elevated temperature to deesterify the N-acetyl groups, thoroughly washed and dried (Knorr, 1984)

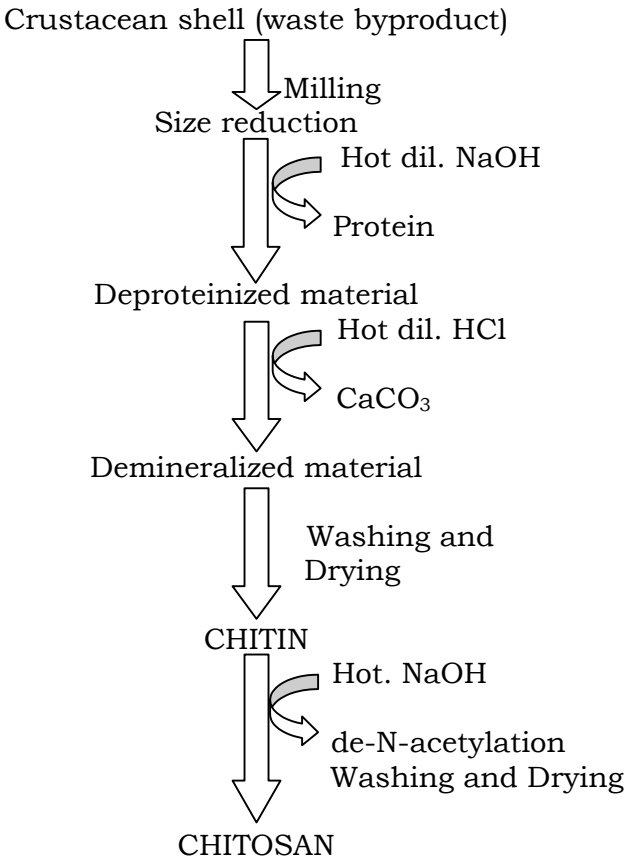


Fig 1.3. Isolation of chitin/chitosan

Properties of chitin and chitosan

Chitin is highly insoluble, of low chemical reactivity, hard, white inelastic, nitrogenous polysaccharide [Muzzarelli, 1974). An important parameter, which influences its physical-chemical and biomedical characteristics, is the degree of N-acetylation especially, in chitosan i.e. the ratio of 2-acetamido-2-deoxy-D-glucopyranose to 2-amino-2-deoxy-D-

glucopyranose structural units. This ratio has a striking effect on its solubility and solution properties. Chitin is N-deacetylated to such an extent that it (chitosan) becomes soluble in dilute aqueous acetic acid and formic acid. Converting chitin into chitosan lowers the molecular weight, changes the degree of N-acetylation, and thereby alters the net charge distribution, which in turn influences the degree of agglomeration. The weight –average molecular weight of chitin is 1.03 to 2.5 x 10^6, but upon N-deacetylation it reduces to 1.0 to 5 x10^5 (Lee, 1974).

Solvent and solution properties

Both cellulose and chitin are highly crystalline, insoluble materials and only a limited number of solvents are known to solubilize them. Chitin/ chitosan degrade before melting, which is typical of polysaccharides with extensive hydrogen bonding. This makes it necessary to dissolve chitin and chitosan in an appropriate solvent system to impart better functionality (Rathke and Hodson, 1994; Ravikumar, 1999).

Application of chitin/ chitosan

Because of their diversified range of applications, both chitin and chitosan are undoubtedly the biomolecules of very great potential. By suitable chemical or enzymic modification, polymer grafting or selective depolymerization several avenues may be realized for tremendous value addition to the basic raw material (see Fig.1.4). As tThey are essentially derived from replenishable resources, biodegradable and therefore do not pollute natural environment; biocompatible not only in animal but also in plant tissues; nontoxic; biologically functional, probably also due to their ability to exhibit polymorphism (changes in crystallinity). Biocompatibility of chitosan allows its use in various biomedical applications (Tharanathan, 2003).

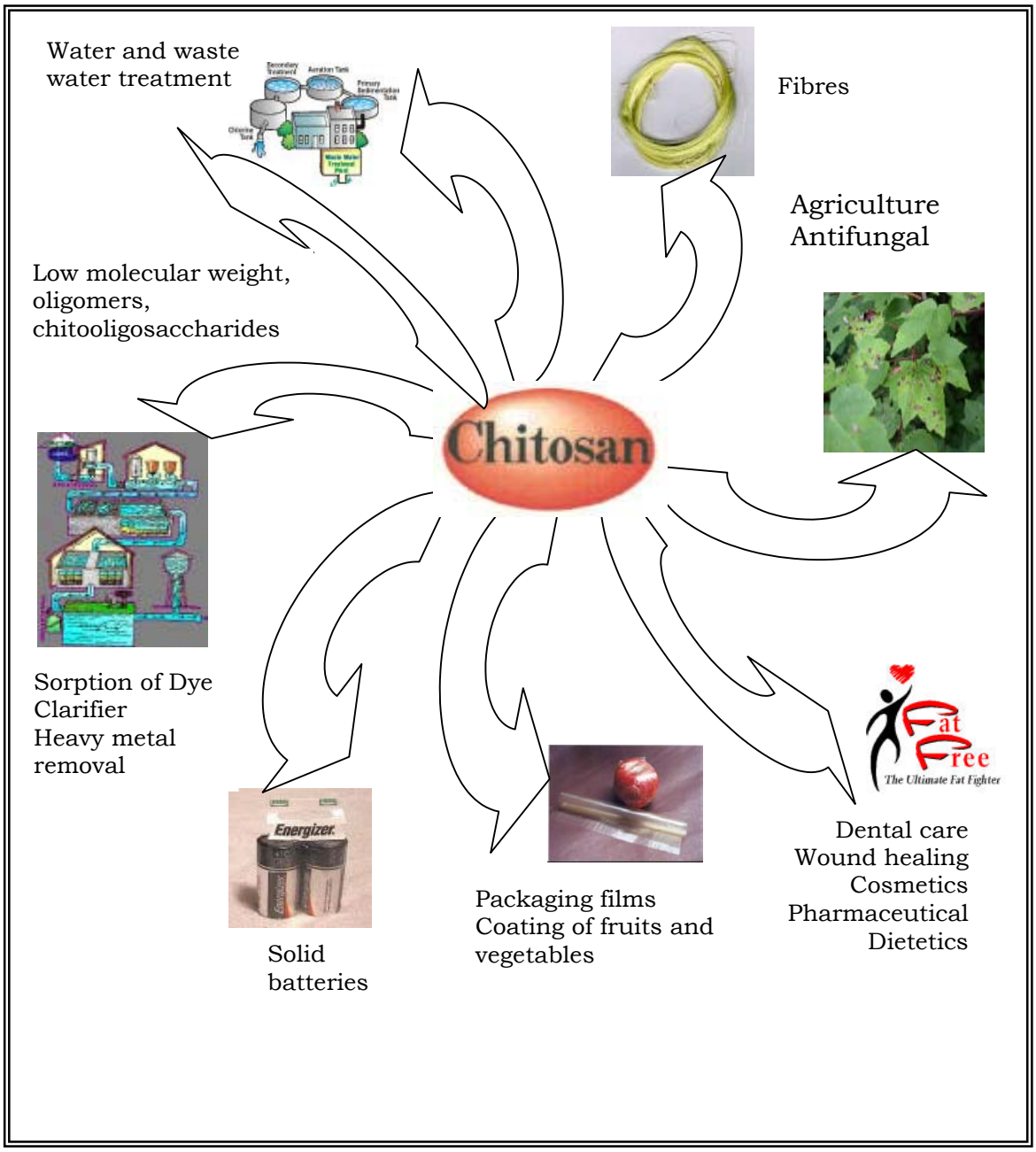


Fig 1.4. Possible avenues for value addition of chitosan

Water and waste- water purification

As environmental protection is becoming an important global concern attention is focused on the development of technology, which does not cause

environmental pollution. The largest single use of chitosan is the clarification of water and waste-water. Alarming awareness of the ecological and health problems associated with heavy metals and pesticides and their accumulation through the food chain has promoted the need for the purification of industrial water prior to its discharge for use (Jeuniaux, 1986; Knorr, 1984). The ability of the free NH_2 groups of chitosan to form coordinate/covalent bonds with metal ions is of greater interest (Immizi *et al.,* 1996). Chitosan powder and or chitosan dried films are of considerable use in metal ion complexing because it will have most of its amino groups free above the pKa value. Use of chitosan for potable water purification has been approved by the United State Environmental Protection agency (USEPA), up to a maximum level of 10 ppm (CRC) (Knorr, 1984). Chitosan, carboxymethyl chitosan and cross-linked chitosan have been shown to be effective in removing mercury, cadmium, Cu^+, Hg^+, Ni^+ and Zn^+ from water, waste water and industrial effluent [Mckay *et al.,* 1989; Muzzarelli, 1977). Due to its unique molecular structure, chitosan has an extremely high affinity for some classes of dyes, including disperse, direct, reactive, acid vat, sulfur and naphthol-dyes (Ravi kumar *et al.,* 1998). Chitosan is also used in the separation of colloidal and dispersed particles from food processing wastes (Green and Karmer, 1979; Kargi and Shuler, 1980).

Paper finishing

Chitosan has been reported to impart wet strength to paper (Allan *et al.,* 1972). Hydroxymethyl chitin and other water–soluble derivatives are useful end-additives in paper making. Use of chitosan in paper making also imparts better paper finish characteristics (Ravikumar, 2000).

Solid-state batteries

For solid-state proton conducting batteries, source of proton–conducting polymer is needed. Chitosan is a biopolymer, which can provide ionic conductivity when dissolved in acetic acid. The conductivity is due to

the presence of protons from the acetic acid solution. The transport of these protons is thought to occur through many micro voids in the polymer since the dielectric constants from piezoelectric studies are small. The choice of a more suitable electrode material may produce a better battery system (Arof *et al.,* 1995).

Cosmetics

For cosmetic applications, the fungicidal and fungistatic properties of chitin and chitosan are made use of. Chitosan is the only natural cationic gum that becomes viscous on being solubilized in acid. Chitosan is used in creams and lotions and some of its derivatives have also been used in the preparation of nail lacquers (Mark *et al.,* 1985). Depolymerized chitosan and carboxymethylchitin are being used as active ingredients of hair shampoo, conditioner, and treatment, because their aqueous solutions are viscous, film forming, and moisture retaining, and give hair and skin softness. (Gross *et al.,* 1982).

Control of enzymatic browning in fruits

Phenolic compounds, together with the activity of polyphenol oxidase, are responsible for browning, which affects the colour, taste, and nutritional value of fruits and vegetables (Huanpu *et al.,* 2001; Kader, 1986). Application of chitosan-coating or film on litchi fruit delayed enzymatic-browning changes, thus altering the contents of anthocyanins, flavonoids, and total phenolics (Zang and Quantick, 1997). It was also shown to delay an increase in polyphenol oxidase activity and partially inhibit increase in peorxidase activity (Shahidi *et al.,* 1999).

Clarification and deacidification of fruit juices

Chitosan salts, which carry a strong positive charge, have been used to control acidity of fruit juices (Imeri and Knoor, 1998). Chitosan has been shown to be a good clarifying agent for grape fruit and apple juice either with

or without pectinase treatment, which gives zero turbidity products with 0.8 kg m^{-3} of chitosan (Soto-Perlata *et al.,* 1999).

Packaging films

The use of edible films and composite coatings to extend the shelflife and improve the quality of fresh, frozen and fabricated foods has been examined during the past few years (Kester and Fennema, 1986; Labuza and Breene, 1989). Due to its excellent film forming properties, chitosan has been used as food wrapping material. The use of N,O-carboxymethyl chitosan films to preserve fruits over a long period has been approved in both Canada and USA (Davies *et al.,* 1989). Due to its ability to form semi permeable film, chitosan can be expected to modify the internal atmosphere as well as decrease transpiration loss and delay the ripening of fruits (Kittur *et al.,* 1998).

Pharmaceutical

Chitosan has been of much use in the pharmaceutical industry for a variety of biomedical applications (Shu *et al.,* 2001). Its polymeric cationic character along with its possession of potentially reactive functional groups have given it unique possibility of utilization in controlled drug release therapy (Nagai *et al.,* 1984; Baba *et al.,* 1989) The drug, either physically blended or covalently linked to the amino groups of chitosan, generally is released from the chitosan matrix after contact with body fluid. Chitosan membranes, utilized as artificial kidney membranes, possess high mechanical strength in addition to permeability to urea and creatinine. Additionally they are impermeable to serum proteins and they probably might be unique in offering the advantage of preventing immission of toxic metals into the blood stream, as it currently happens using other artificial membranes. N- and O-sulfated chitosans have been found to possess 15 to 45% of the anticoagulant activity of heparan *in vitro* (Whistler and Kosik, 1971).

Covalently and ionically cross-linked chitosan hydrogels are shown to exhibit innumerable medical applications although their use in humans is yet demonstrated to be risk-free. Ionically cross-linked chitosan hydrogel offer more possibilities as drug-delivery systems, although they lack mechanical stability (Ravikumar, 2000)

Chitosan processes all the desirable characteristics required for making an ideal contact lens, exhibiting optical clarity, mechanical stability with sufficient optical correction. Contact lenses are made from the partially depolymerized and purified squid pen chitosan, and they are clear, tough, and bear good tensile strength and tear strength (Markey and Bowman, 1989).

Due to its excellent biocompatibility with the human body tissue, chitosan was found to be effective for all forms of skin dressing, suture thread in surgery, as implants or gums cicatrisation in bone repair or dental surgery. In dental creams, it extends the paste shelflife and also helps in regenerating the gums that are defective.

Agriculture

Chitosan and its derivatives exhibit plant protecting and antifungal properties, triggering defensive mechanism in plants against infection and parasite attack, even at a very low concentration. It is used in the form of solution or as coating of the seed. Chitosan acts at several levels, by strengthening the root system and thickening the stem. It also behaves like fertilizer by accelerating the germination and growth of plants (Ravikumar, 2000).

Dietetics

Chitosan is not digestible in human body; it behaves like a dietary fibre. But above all it is an excellent fat trapper, by precipitating liquid fat in the

intestine and thereby reducing the rate of cholesterol absorption by 20-30% (hypocholesterolemic agent) (Yogesh and Sachin, 2002).

Aim and scope of the present investigation

Plastics have become part and parcel of our everyday life and the plastic industry has emerged as a rapidly expanding industry in the past several decades. The treatment of waste plastics has become a serious problem because of the difficulty of ensuring reclaimed land and burning by incineration. Their total non-biodegradability as well as an increased environmental consciousness by the consumers and Government bodies has paved the way to look for alternative approaches. This development has for the best part been focused on alternative packaging films derived from natural biopolymers which are replenishable and completely biodegradable under a variety of ecological systems. Biopolymer films are generally prepared by using biological materials such as polysaccharides, proteins and their derivatives, which are naturally and abundantly available. Natural biopolymeric films have the advantage over synthetic biopolymers since they are totally biodegradable and are derived from renewable raw materials. They can be used effectively as an alternative to synthetic plastics. Biopolymers have also desirable overall mechanical and barrier properties.

Chitin/chitosan are value added byproducts recovered from the seafood industry wastes. Only very scanty information is available on the use of chitosan as a packaging material. Chitosan films are prepared by drying at ambient temperature for 30-36 hr, after spreading the solution over a leveled glass plate. As this is a time consuming process, a simple drying method was felt desirable for preparing chitosan films. In the present investigation an attempt has been made to cast the film under Infrared heating and to design a continuous wet casting protocol unit. Attempts have also been made to study the characteristics of chitosan-based packaging films produced using different plasticizers and their application potential in extending the shelflife.

Thus, the main objective of this investigation was to develop an experimental design protocol unit for preparing native and modified chitosan films and to look for their physico-chemical, mechanical, barrier and functional properties including biodegradability characteristics. Accordingly, the following work plan was proposed to be carried out.

1. Preparation of chitosan films under different drying conditions and to compare their mechanical and barrier properties

2. Modification of the film properties by adding plasticizers and other additives,

3. Shelflife extension studies of fruits, vegetables and dairy-bakery products using chitosan films, and

4. A study of antimicrobial, antifungal properties of chitosan films.

Introduction

In 1936, Rigby was granted a patent for making film from chitosan and a 2nd patent on making fibers from chitosan. The films were described as flexible, tough, transparent and colourless with tensile strength of about 6210 kPa. Muzzarelli *et al.* (1974) showed that film forming qualities of chitosan are dependent on the structure. The mechanism as well as the prediction of water transport through hydrophilic films are extremely complex, due to nonlinear water sorption isotherms and water content dependent diffusivities (Swartzberg, 1986).

Chitosan films have been proposed for use in food processing (Bai *et al.,* 1988), membrane separation (Aiba *et al.*, 1986), chemical engineering, medicine and biotechnological areas (Senstad and Mattiassion, 1988). The mechanical properties, permeability, thermal decomposition points, solvent stability, etc., are parameters considered vital for selection of right film for specific applications (Collons *et al.,* 1973). Factors influencing affecting the selectivity of the film are numerous,viz. membrane pore size (Crig, 1970), swelling index (Mochizuki *et al.*, 1989), film making conditions (Kienzle-sterzer *et al.,* 1982; Aiba *et al.,* 1986; and Hwang *et al.,* 1986), thickness (Nakatsuku and Andrady, 1992), casting method (Ogawa *et al.,* 1992; Samuels, 1981), and solute characteristics such as molecular weight, and solvent used (Blair *et al.*, 1987). Biocompatability is another parameter of importance in biomedical field. Chitosan membrane is not antigenic in a mammalian test system and it is non-thrombogenic (Muzzarelli, 1977). It is very suitable for use as an artificial kidney membrane (Hirano and Noishiki, 1985) and in drug delivery systems (Sawayanagi *et al.,* 1982). Chitosan films are prepared by dissolving chitosan in dilute acid and spreading on leveled surface and air-drying at room temperature. Films are also prepared by drying at 60° C in an oven by spreading the solution on polypropylene film plexi glass (Butler *et al.*, 1996, Wiles *et al.,* 2000). All these processes are time consuming. Hence, an attempt was made to prepare chitosan film at

shortest time using Infrared system for drying. Some trials were also made to develop a continuous prototype-filming unit to prepare chitosan films.

Infrared heating

Infrared heating offers many advantages over conventional drying methods, and involves the exposure of a material to electromagnetic radiation in the wavelength region 1.8-3.4 μm, which facilitates the water molecules to vibrate at a frequency range of 60,000-150,000 MHz and allows for rapid internal heating and a rise of water vapour pressure inside the material with subsequent evaporation and drying (Ginzburg, 1969). Alcantara *et al.* (1998) reported drying rate effect on the properties of whey protein films and observed that films dried at higher temperature showed stiffer, stronger and less extendable properties than films dried at lower temperature. In an earlier study, whey protein and soy protein films were prepared under microwave drying at higher temperature and their properties were studied (Kaya and Kaya, 2000). Donhowe and Fennema (1993a) have reported the properties of methylcellulose films prepared at elevated temperature. Little information is available on the molecular mechanisms of the processes of forming films (Chen, 1996).

This chapter deals with characterization of raw materials, selection of media to dissolve chitosan and base material, drying conditions, properties of films, sorption curves, WVTR at different RH. Response surface methodology was used to find the optimum values for tensile strength (TS), percent elongation (%ε) and modulus of elasticity (ME) at different storage temperature, relative humidity (RH) and storage period.

Materials and Methods

Crude chitosan (CH1) was obtained from CFTRI Regional Center at Mangalore and later a purified sample (CH2) was obtained from M/s Sea Food Industry, Cochin, Kerala. Chitosan (10 g) was treated with 40%

sodium hydroxide (100 ml of water) in water bath at 100^0 C for 60 min, filtered, later chitosan was water washed thoroughly and dried in an oven. The dried samples were taken for characterization. Chitosan was soluble in dilute acids and the concentration of acid used was dependent on its molecular weight and degree of deacetylation.

Bulk density

The bulk density of chitosan powder was determined following the procedures of Wang and Kinsella (1976) and Anderson *et al.,* (1978). Briefly, one gram of chitosan sample (80-100 mesh size) was placed in 15 ml tapered graduated centrifuge tubes, vibrated on a vortex mixer for one min, and packed by gently tapping the tube on the bench top repeatedly. The volume (V) of the sample was recorded. The procedure was repeated two times for each sample, from the weight of the tube (W1) and weight of the empty tube (W2), the bulk density was expressed as grams per ml of the sample.

$$\text{Density} = \frac{W1-W2}{V} \text{ g/ml}$$

Molecular weight determination

1) By Viscosity method

Chitosan (1g) was dissolved in 90 ml of acetate buffer solution [sodium acetate, 0.2M + acetic acid, 0.5M]. The solution was filtered using glass wool and then made up to 100 ml in a volumetric flask using buffer solution. 0.1 % to 0.5 % chitosan solutions were prepared from the stock solution, taken in an Ostwald viscometer, and the time taken by the solution to travel between 2 markings was noted. A graph of $\eta Sp./C$ versus C was plotted. The molecular weight was determined by using Mark-Houwnik relation.

$$[\eta] = K^a [M]\alpha$$

where, [η] -Intrinsic viscosity obtained from the graph, K & α - constants and their values are 3.5×10^{-4} and 0.76, respectively and M- molecular weight (Kittur *et al.*, 1998, Rinaudo *et al.*, 1993)**.**

2) By Gel permeation chromatographic (GPC) method

In this method, Sepharose CL-2B column (bed volume 180 ml) was used. Acetic acid (0.15 M) + sodium acetate (0.35 M) was used as the eluent. The GPC column was calibrated with chitosan and dextran standards and the void volume was determined using dextran T-2000 (M_W 2000 kDa). The eluted fractions (1.6 ml) were analyzed by phenol-sulphuric acid method (Ohno and Yatamae, 1956)

Preparation of chitosan films

Chitosan (2% w/v, Mw 100 kDa, Degree of acetylation ~20%) was dissolved in water containing 1% acetic acid by constant stirring and the viscous solution was filtered through sieve No.80 having square opening to remove any undissolved impurities, and later the solution was degassed by using vacuum pump to remove the entrapped air. Approximately 200 ml of chitosan (2%) solution was poured on a glass plate (21 x 29 cm) and dried at ambient or oven or by infrared method (Tharanathan *et al.,* 2002). After drying, the films were peeled off and stored at ambient condition (25 °C, RH of 50 %) for 48 hr. For further preparation of films Polyester base material was used. For casting films the optimum concentration of chitosan used was 2 g (CH1) and 1 g (CH2), and acetic acid required for its complete dissolution was found to be 1.0 and 0.5%, respectively. At lower concentration, acetic acid and chitosan form a gel due to improper solubility, if chitosan concentration is decreased the strength of the film also decreases.

Density measurements

Density of the film was determined using a flotation method at 25 °C using CCl_4 (1.5935 g/ml) and heptane (0.71 g/ml) as solvents. The film (1.5 × 1.5 cm) was immersed in 5 ml heptane taken in a small beaker. CCl_4 was taken in a burette and added dropwise to the beaker until the film floats in the middle of the solution, and density of the film was calculated by using the formula (Qurashi *et al.,* 1992)

$$\text{Density} = \frac{V1d1+V2d2}{V1+V2} \quad \text{g/ml}$$

Where, V1- volume of heptane in ml, V2-volume of CCl_4 in ml, d1-density of heptane in g/ml, d2-density of CCl_4 in g/ml

Thickness

Film thickness was measured with a constant load micrometer (Testing Machines, Minneapolis, USA). Five thickness values were taken along the length of the filmstrip and the mean value was used for tensile strength calculation. Similarly, five measurements were taken on each WVTR and OTR samples, one at the center and four around the perimeter and mean values were used for calculation.

Colour

Colourimetric method (CR-Minolta, Minolta Camera Co. ltd, Japan) was used to determine L, a, b and opacity values. The instrument was standardized with white plate supplied by the manufacturer.

Tensile strength (TS), % elongation (%ε) and Modulus of elasticity (ME)

LLOYDS Universal testing (LLOYDS - 50K, London, UK) instrument was used to measure tensile strength (TS) and percent elongation (%ε) at break. The tests were carried out according to ASTM D-882 standard test (ASTM, 1995a), with initial grip separation of 50 mm and cross head speed of 50 mm/min. TS was calculated by dividing the maximum load for breaking film by cross sectional area, %ε by dividing film elongation at rupture to initial gauge length, and the values were measured both in longitudinal and transverse directions to observe whether any difference in the orientation of polymer chain occurs. Percent elongation is the ratio of extension to the length of the sample. The ME is the ratio of stress to strain at the linear portion of the curve. All means were compared with each other, the results of ANOVA were indicating significance ($p<0.05$). TS of the films was measured both in Machine direction (MD) and Transverse direction (TD).

Tear strength

The internal tearing resistance (in Newton, N) was determined as per TAPPI standard test method (ASTM, 1988) using LLOYDS Universal testing (LLOYDS - 50K, London, UK) instrument. This involves the determination of force necessary to propagate a tear in the specimen. The specimen was cut into a size of 120 mm length and 25 mm width. The specimen was cut longitudinally up to 70 mm. One edge of the cut specimen was fixed to upper jaw and other one was fixed to lower jaw of the instrument. The speed of the specimen was set at a rate of 50 mm/min.

Burst strength

This test measures the ability of film to withstand pneumatic load, and gives a sort of combined tear and tensile properties. For the present study pneumatic burst strength tester was used according to ASTM 1980a. The sample free from creases was placed in position and clamped firmly. The tester was connected to the compressed air pipeline, fitted with the two

gauges and open valve to suit pressure requirements. A steady pressure at the rate of 680-690 kPa was allowed to pass inside until the specimen ruptures and burst pressure was recorded.

Impact strength

The films were tested for impact strength as per ASTM methods. The normalized impact strength was calculated by dividing the impact energy by the average film thickness.

Barrier properties

A. Water vapor permeability (WVP)

WVTR of films was determined using aluminum dishes according to ASTM E-96-97 method (ASTM, 1980b). Films with an exposed area of 50 cm^2 were tested at 90% RH in a humidity cabinet (Laboratory Thermal Equipment, Glasgow, UK). Weight loss graphs were plotted with respect to time, and linear least-square method used to calculate water vapor transmission rate (WVTR) as per the following equation (Chinnan and Park, 1995)

WVTR = slope/film area ----(1)

Water vapor permeability (WVP) was determined (Chinnan and Park, 1995) by using the equation (2)

WVP= (WVTR/ P2-P1)x L ----(2)

where P1 the a partial pressure (kPa) inside the cup, and P2 the water vapor partial pressure (kPa) at the film outer surface in the film system. L is the average film thickness (mm).

B. Oxygen transmission rate (OTR)

Oxygen transmission rate (OTR) was determined using volumetric permeability cell (Customs Scientific Instruments, New Jersey, USA) according to ASTM D-1434 procedure (ASTM, 1983). The test makes use of permeability cell consisting of two stainless steel discs that form cylindrical cavity when the discs are superimposed. The film to be tested was clamped between the two discs using six equally spaced bolts after placing filter on the upper discs (as support) and a rubber gasket to ensure a pressure tight fit. The cell consists of a glass capillary in a vertical position to an opening in the center of the upper disc. Suitable gas inlet and vent lines were provided on both sides of the cell. Oxygen was supplied from surge tank at a constant pressure to the bottom of the cell. A short plug of mercury contained in a capillary was displaced upward by the permeating gas and this displacement gives the rate of permeation of the gas through the packing material.

An electro-mechanical vibrator is used to avoid friction to the movement of the plug. The change in volume of the permeates measured as a function of time and the displacement of mercury v/s time is plotted and slope of the straight line is obtained. Then OTR is calculated using the formula.

$$OTR = \frac{26133.90 \times \text{Slope}}{\text{Pressure}} \text{ cc/m}^2\text{/day atmosphere}$$

where 26133.90 is capillary constant.

X-Ray diffraction

X-Ray diffraction patterns of chitosan films were obtained by using a EG-7G solid state germanium liquid nitrogen cooled detector Sintag XDS-2000 instrument equipped with a θ-θ goniometer, under the following operating conditions: 30 kV and 25 mA with CuKα1 radiation at λ 1.54184 Å. The relative intensity was recorded in the scattering range (2θ) of 4-40°,

and crystallinity index (CrI) was determined as per the method of Focher *et al.,* 1990, by using the equation $CrI = I_{110} - I_{am} / I_{110}$ where I_{110} is the maximum intensity (2θ, 20°) of the (110) lattice diffraction and I_{am} is the intensity of amorphous diffraction (2θ, 16°).

Differential scanning calorimetry (DSC)

The samples were analysed using mettler DSC 30 Switzerland equipment is supported by thermal software on a Compaq computer, which is pre-calibrated. The accurately weighed (5 mg) material was placed in an aluminum cup and hermetically sealed. Empty sealed cup was used as reference and runs were performed in duplicates. Analyses were done under continuous flow of dry nitrogen gas (10ml/min) at a heating rate of 10^0 C.

Fourier transform infrared spectroscopy (FTIR)

IR spectra in the range 3200-1000 cm^{-1} of samples were scanned in FTIR spectrometer (Perkin-Elmer spectrum 2000 USA) under dry air at room temperature. Samples were preconditioned by keeping in 0% RH desiccators.

Scanning electron microscopy (SEM)

The dried films samples thus obtained were spread over double-sided conducting adhesive tape pasted on a metallic stub and coated with gold (100 μ) in a sputter coating unit for 5 min and observed under Scanning electron microscope (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK) at 20 kV.

Thin layer drying model

The drying phenomena of biological products during the desorption period is controlled by the mechanism of liquid diffusion. This can be explained by thin layer models. The drying curves were obtained by plotting

a graph of moisture ratio versus time. The moisture ratio (MR) is defined by Page's equation (Page, 1949),

$$MR = \frac{X_{db} - X_e}{X_o - X_e} \qquad \text{----(3)}$$

Where X_{db} =moisture content at any time (t), X_e = equilibrium moisture content, and Xo = initial moisture content.

The equilibrium moisture content X_e of the dried product could be taken as its final moisture content X_f, which is reported to be more realistic from the practical stand point of view (Brooker *et al.,* 1981). A similar assumption has also been made by several authors (Weller and Bunn, 1993; Ramesh and Srinivas Rao, 1996; Ramesh *et al.,* 2001; Ren and Chen, 1998). Hence, the modified equation for MR could be,

$$MR = \frac{X_{db} - X_f}{X_o - X_f} \qquad \text{----(4)}$$

During thin layer drying with any method of heating (convective, conductive or radiative) the MR can be correlated with the drying time by using a quadratic form as indicated below.

$$MR = Ax^n + Bx^{n-1} + Cx^{n-2} \ldots\ldots\ldots\ldots\ldots\ldots + D \qquad \text{----(5)}$$

The analysis of the drying data revealed that for OD, which is convective, the value of n was 1 and for ID, which is radiative n was 2. With this the equation reduces to

For ID, $MR = Ax^2 + Bx + C$ ----(6)

for OD, $MR = Bx + C$ ----(7)

where A,B,C are constants based on drying time (t), and temperature (T)

Model development and Statistical analysis

The experimental MR and time values were analysed using nonlinear regression to determine the coefficients, and were correlated with drying temperature. Regression analyses were done using Microsoft Excel routine. The coefficient of correlation (R^2) was determined by plotting M_{exp} versus M_{pre}, and root mean square error (RMSE) was determined.

$$RMSE = \sqrt{\left[\frac{\sum (M_{exp} - M_{Prd})^2}{N}\right]} \qquad \text{----(8)}$$

Standard deviation of difference (SD) is given by,

$$S_D = \sqrt{\left[\frac{\sum (M_{exp} - M_{Prd})^2 - \frac{\left(\sum (M_{exp} - M_{Prd})\right)^2}{N}}{N - 1}\right]} \qquad \text{----(9)}$$

The average percent error (E) is given by:

$$E = \frac{100}{N} \sum \frac{\left|M_{exp} - M_{Prd}\right|}{M_{exp}} \qquad \text{----(10)}$$

where M_{exp} = experimental moisture content, M_{prd} = predicted moisture content, N = number of trials.

Based on the multiple regressions the expression to estimate the MR at any time (t) and at any temperature (T) during drying (OD and ID) process was determined.

Response surface methodology

To study the effect of relative humidity (RH), storage temperature (T) and storage period (D) on the response parameters such as tensile strength (TS), percent elongation (%ε) and modulus of elasticity (ME), below mentioned methods are used.

Experimental design and data analysis

The experimental design chosen for this study was that of Box and Behnken, a fractional factorial design for three variables (Montgomery, 1997). The design was preferred because relatively few experimental combinations of the variables are adequate to estimate complex response functions. Three levels, such as low, medium and high are denoted as -1, 0 and +1 in coded level of variables, were employed to fit a full quadratic response surface model and approximate the factor levels that provide the optimal response.

The design of the experiment is presented in Table 2.1. Three duplicates are included at the center of the design. The total number of test runs needed for this design was 15, which is less than that required for central composite or 3 x 3 factorial design. The experimental conditions were selected for each variable based on prior studies. Experiments were carried out according to the design points with independent variables such as temperature (X_1), relative humidity (X_2),) and storage conditions (X_3).

Response surface methodology was applied to analyse the effect of independent variables on response parameters. In such method the responses studied (Y) are matched to the code factors (x_i, I=1, …,) by the following polynomial model associated with experimental design (Khuri and Cornell, 1987)

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n} b_{ij} x_i x_j \quad \text{----[11]}$$

Table 2.1. Box-Behnken experimental design for the independent variables (actual and coded levels).

Expt. No	Independent variables					
	Actual level			Coded level		
	Temp.	RH	Days	Temp	RH	Days
1	4	40	6	-1	-1	0
2	50	40	6	1	-1	0
3	4	80	6	-1	1	0
4	50	80	6	1	1	0
5	4	60	3	-1	0	-1
6	50	60	3	1	0	-1
7	4	60	9	-1	0	1
8	50	60	9	1	0	1
9	27	40	3	0	-1	-1
10	27	80	3	0	1	-1
11	27	40	9	0	-1	1
12	27	80	9	0	1	1
13	27	60	6	0	0	0
14	27	60	6	0	0	0
15	27	60	6	0	0	0

Co-efficients b_0, b_i and b_{ii} represent the constant, linear and quadratic effects and a_{ij} represents the interaction effect of code factor x_i. Statistical package Statistica'99 (StatSoft, USA) was used for regression and ANOVA analysis. Response surface graphs were obtained from the regression equation in actual levels of variables, keeping the response function on the Z axis with X and Y axes representing the independent variables while keeping the other

variable constant at the center (corresponding to 0, 0 coded level) points. The results were validated and confirmed by carrying out the experiments with values, which were not the design points.

Simultaneous optimization

Canonical analysis (Myers, 1971) was performed on the predicted quadratic polynomial model to examine the overall shape of responses and to characterize the nature of the stationary point. Optimization of the response function consists of its transition from the origin to the stationary points. The response function was expressed in terms of the new variables, the axes of which correspond to the principal axes of the contour system. The roots of the auxiliary equation were calculated initially to know the nature of optimum. The response would be maximum if all the roots show negative values, and minimum if all the roots show positive values and if they show a combination of positive and negative values, it represents a saddle point or minimax. Simultaneous optimization was done according to the method suggested by Derringer and Suich (1980), where all the individual desirability functions were combined into an overall desirability function, which is defined as the geometric mean of individual desirability functions. Higher the desirability value more desirable system.

Results and Discussion

Chitosan, a versatile biopolymer has a wider application in various fields. The chitosan sample (CH1) initially obtained from the CFTRI regional center at Mangalore, was light yellow with a degree of deacetylation of 60%, which was further deacetylated by hot alkali treatment, whereas the chitosan sample (CH2) obtained form M/s India Sea Food, Cochin, was whitish yellow. The nature of two samples is tabulated in Table 2.2.

The density did not vary much, the value was found to be 0.35g/ml for the both samples. Molecular weights of CH1 and CH2 samples were

determined by viscometry and confirmed by GPC method. Viscometry is one of the simplest and rapid methods for determining the molecular weight.

Table 2.2. Characterization of chitosan samples

Properties	CH1	CH2
Nature	Flakey	Powder
Colour	Dark yellow	Light yellow
Degree of deacetylation	85%	90%
Molecular weight (Da)		
a) Viscometry	1,05,000	2,02,000
b) GPC	1,00,000	2,01,000
Intrinsic viscosity	2.75	3.74

Molecular weight was found to be 1.05 and 2.02 kDa, respectively. In GPC the molecular weight was found to be 1.00 and 2.01 kDa, and no significant differences were found between the two the methods.

The FTIR spectra of the CH1 and CH2 are shown in Fig.2.1. The

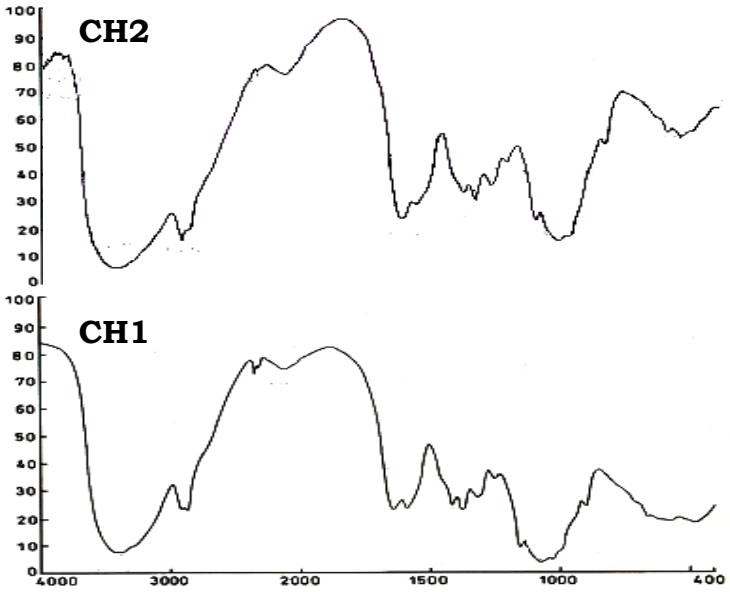


Fig. 2.1. FTIR of chitosan samples

bands occurred at 1655 cm^{-1} (amide vibration mode) and 3265 and 3100 cm^{-1} (NH band stretching). The degree of deacetylation (DD) determined by absorbance at 1655 and 3455 cm^{-1}, of CH1 and CH2 was found to be 85% and 90%, respectively. The region 1500-1200 cm^{-1} is related to local Symmetry and the band around 1429 cm^{-1} assigned to CH_2 bending is dependent on the most favorable orientation of primary hydroxyl group. The vibration at 1379 cm^{-1} has been assigned to CH bending along with some OH bending contribution. The ratio of absorbances at 1388 cm^{-1} and 2900 cm^{-1} gives an index of crystallinity.

X-ray diffraction is used to study the existence of polymorphism. Polymeric forms of a compound have different crystal structure and therefore should have distinct powder X-ray diffraction patterns. Diffraction patterns of the two-chitosan samples (Fig. 2.2) showed characteristic peaks at 20˚ indicating some degree of orientation of polymer chains. The peak around 9˚

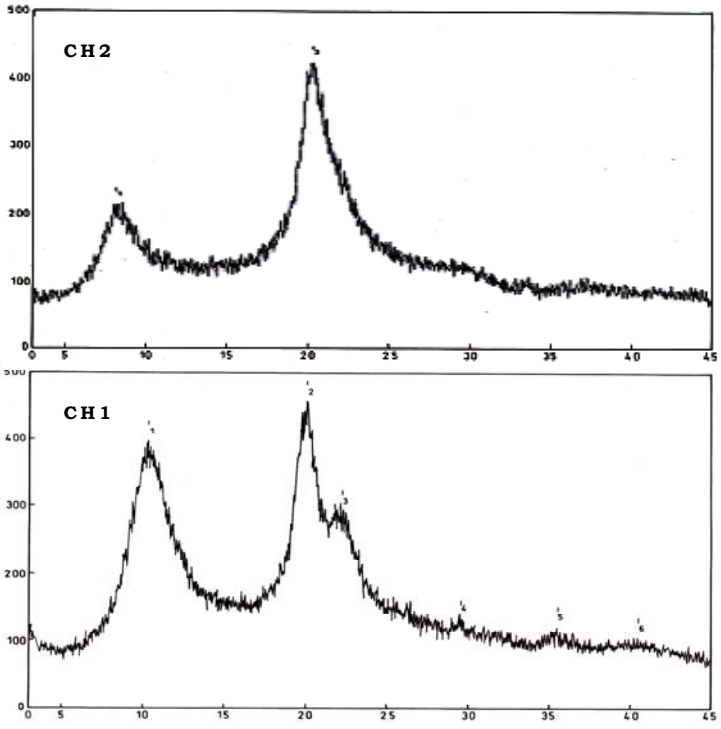


Fig. 2.2. X-ray diffractogram of chitosan samples

suggests the presence of hydrated crystals and crystals of α-chitin chain segment (Lee, 1999).

Selection of suitable base material to cast films

Literature reports show that chitosan films are cast by dissolving chitosan in solvents like acetic acid, lactic acid, formic acid, propionic acid or hydrochloric acid, pouring the solution on levelled glass plate and later dried at ambient temperature for 30-36 hr. The films were peeled carefully from one end. Initially work was done on casting chitosan films on different base materials such as, glass plate (Kittur *et al.*, 1998;Hasegawa *et al.*,1992), plexi glass (Wong *et al.*, 1992) and teflon coated surfaces (Park *et al.*, 2002). It was difficult to remove the film from glass surface without breaking, whereas in Teflon coated surface the films were observed to be drying only at from the edges, and films showing lot of wrinkles. Additionally, stainless steel, aluminum sheet and polyester sheets were tried to get better films at room as well as at higher temperature. At room temperature all these gave films, which could be easily removed. Similar trials were done under different drying methods i.e. oven drying and infrared drying. Table 2.3. shows the

Table 2.3. Effect of base materials on quality of chitosan films

Base Materials	Drying temperature (° C)	Film thickness (gauge)	Remarks
Glass	80-100	ND	Difficult to peel
Petri dishes	80-100	ND	Difficult to peel
Stainless steel	80-100	150-200	Coloured films
Aluminium	80-100	150-200	Coloured films
Teflon	80-100	150	Wrinkles
Polyester sheet	80-100	50	Good films, easy to peel off

nature of chitosan films obtained at higher temperature. In metal sheets the excessive colour observed was due to heating of the materials. Even though

in Teflon the films were obtained without difficulty, but lot of wrinkles were observed in the films. But in polyester sheets, the films were free from all these demerits and a good quality film was obtained.

Preparation of films with different organic acids

Chitosan when dissolved in organic acids forms viscous solutions, which are used to make functional films. In the present study, chitosan (CH2) was dissolved in acetic acid (AA), formic acid (FA), lactic acid (LA) and propionic acid (PA) at 0.5% level. The initial pH of the acids were 3.23, 2.60, 2.85 and 3.18, which after dissolving chitosan, changed to 4.13, 2.90, 3.70 and 4.43, respectively. Films from each of them were cast as before.

TS values of the chitosan films prepared with four different organic acids, shown in Table 2.4, varied from 18.54 to 48.34 MPa. During film formation hydrogen bonding between hydroxyl groups and amino groups increases with increase in the concentration of chitosan.

Table 2.4. Properties of chitosan films obtained from different acid

Properties		Lactic acid	Propionic acid	Formic acid	Acetic acid
Tensile strength (MPa)	MD	21.9 ± 4.2	31.53± 3.48	48.34 ± 4.28	39.24± 4.65
	TD	10.54 ± 4.5	28.70 ± 2.86	35.61 ± 2.48	36.78± 6.43
% Elongation	MD	37.02 ± 6.34	9.48 ± 2.32	34.62 ± 5.45	11.3 ± 4.32
	TD	30.02 ± 7.23	6.77 ± 1.34	24.61±4.34	6.43 ± 3.21
Modulus of elasticity	MD	145.4 ± 20.48	1546 ± 213	1248 ± 162	1895 ± 245
	TD	89.62 ± 16.24	1362 ± 234	1006 ± 70	1576 ± 234
WVP (g. m/m^2.day.kPa)		0.03 ± 0.012	0.02 ± 0.01	0.015± .002	0.013± .004
OTR 10^{-6} (cc.m/m^2.day.kPa)		ND	140.9± 2.48	98.26±1 4.12	35.26± 3.54

The inter-molecular arrangement of chitosan in an aqueous acidic solution is influenced by characteristics such as ionic strength and degree of

dissociation. The mechanical strength of the film changes with the type of acid used. The result is supported by those of Kienzle-Sterzer *et al.* (1982), who reported that the acid used in preparing film might affect both junction density and topological limitation of film. This may be due to variation in the interaction between chitosan and the respective acid solution.

Among the acids tested, FA resulted with high TS followed by AA, PA and LA. Lactic acid formed significantly weaker films. The result agrees with those of Rhim *et al.* (1998), who found acetic acid giving tougher chitosan films than malic, lactic and citric acids. Park *et al.* (2000) showed that in acetic acid solution, chitosan forms dimers indicating that the intermolecular interaction is relatively stronger.

Earlier work of Park *et al.,* (2000), who reported chitosan films prepared from acetic acid had a TS of 65.96 MPa., where chitosan sample has a viscosity 15 centipoise. Rhim *et al.,* (1998) reported 41.96 ± 5.9 for TS of chitosan films with 1% acetic acid, in partial agreement with the present study. The subtle differences may be attributed to different raw materials and preparation methods used. The percent elongation measures the ability to stretch chitosan films, which also varied according to the type of acid and their interaction, with a mean value varying from 6.77 to 37.02 %. AA and PA films showed a low value compared to LA and FA films. LA films showed greatest %ε values among the films tested. Park *et al.* (2002) determined %ε of chitosan films prepared with acetic acid, citric acid and lactic acid solutions and reported LA film to have the highest value of 31.9 to 104.9%. Generally it is known that there is inverse relationship between TS and %ε of the biopolymer films. That is, extensibility (lower E) of films reduced as strength of films increased (greater TS) (Rhim *et al*, 2000)

ME values show toughness of the films. As the ME values increase the films were more tougher. AA films were more tougher than any other films.

WVP of chitosan films ranged from 0.0131 to 0.03 ± 0.012 (Table 2.4). WVP of AA films showed better barrier to water vapour, while LA films showed lesser barrier. A significant interaction with acids was evident from the OTR values, which varied from 35.36 to 140 in disagreement with Muzzarelli *et al.*(1974), where the values were 7.2 X 10^{-8} cc/m^2.day.atm. AA films showed higher barrier to oxygen.

During solution preparation lactic acid and formic acid were observed to show more entrapped air compared to acetic acid. So casting of the films was easy in acetic acid, which also had comparatively better film properties. For further work acetic acid was used to prepare chitosan films.

Drying curves of chitosan films

The drying of films under infrared heating appeared to be faster compared to other methods of drying. For studying drying curve characteristics, a film thickness of 100 gauge (25 μm), was used. As the temperature increased the drying time reduced (Fig 2.3.) To get a film at 80 ± 2 °C by OD, it took ~120 min, whereas in ID it took less than 60 min, on the other hand at 100 ± 2 °C the drying time was 70 and 25 min, respectively.

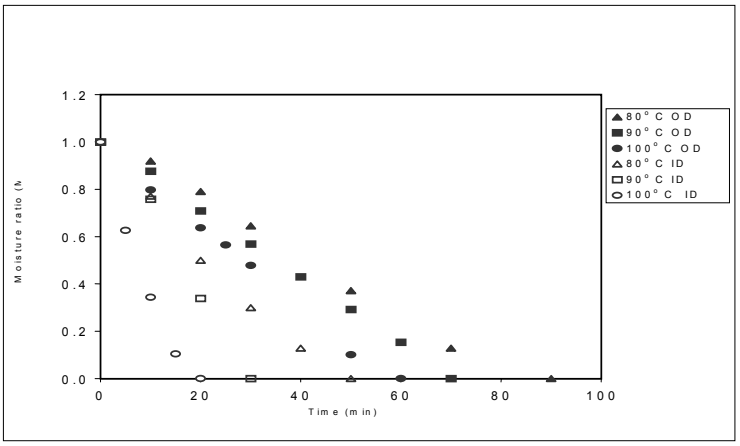


Fig. 2.3. Drying curves of chitosan films prepared under different drying conditions

Thus, the time required to dry the film was reduced by over 50% in ID. At ambient temperature (~27 °C) the time required was 30-36 hr. No entrapped air bubbles were observed when the dried films were peeled off from the surface, contrary to that reported on whey protein films (Alcantara *et al.,* 1998). This may be due to differences in the chemical nature of starting materials (hydrocolloids) used. The moisture content at different intervals during drying over a range of temperature 80-100° C was determined. A graph of moisture ratio (MR) versus drying time is shown in Fig.2.3. The equations correlating time and temperature for both drying methods were determined.

For OD, MR predicted. = -0.000255Tt + 0.008617t +0.09974 --------(12)

For ID, MR predicted. = -0.000075 Tt2 –0.00578t2 -0.0027965Tt
+0.192t+1.004 --------(13)

where T = drying temperature, t = drying time

Using these equations the MR during the two drying methods at various time intervals were predicted and compared with the experimental values. Figs. 2.4. and 2.5. indicates the closeness of prediction. The statistical values indicated in Table 2.5, further confirm the validity of thin

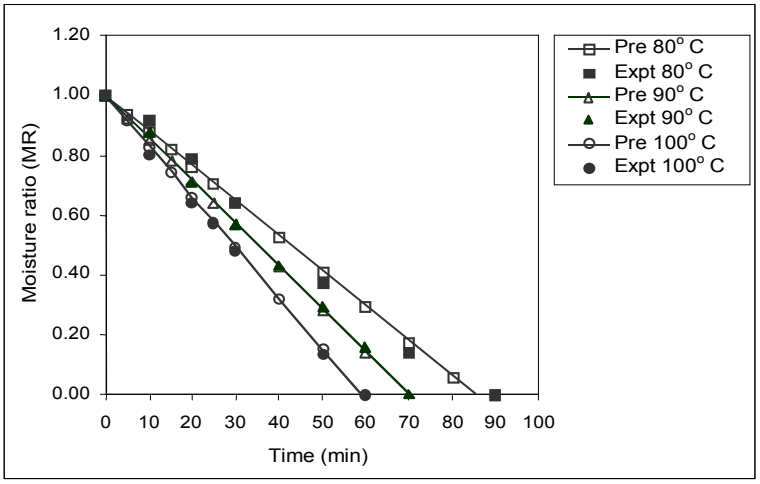


Fig 2.4. Experimental and predicted drying curves for oven dried films

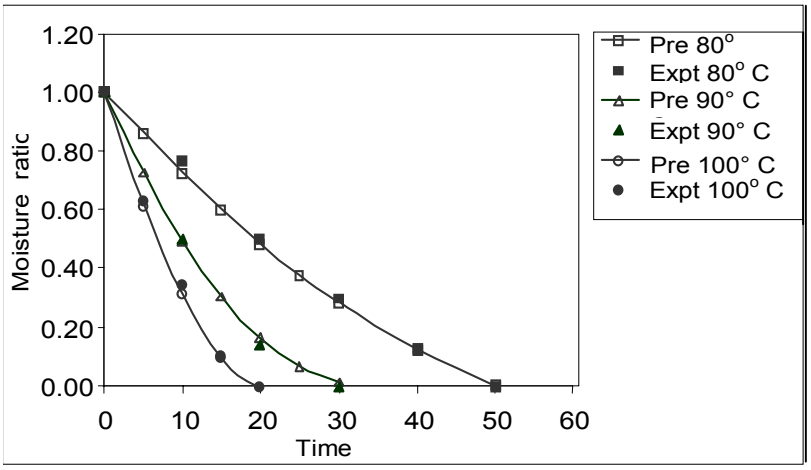


Fig 2.5. Experimental and predicted drying curves for Infrared dried films

layer model for the two drying methods. According to Fick's law, the evaporation rate is proportional to the saturated water vapor pressure immediately above the solution surface. This pressure may be considered as constant throughout the drying process until the final stages of drying, when the pressure will reduce. This means that the last part of MR curve (i.e. when MR<0.10 for the OD method) will be non-linear (Fig 2.4).

Table 2.5. Kinetic constants derived from different drying protocols

Drying Temperature, °C	**Drying method**							
	Infrared (ID)				**Convective (OD)**			
	RMSE	E	SD	R^2	RMSE	E	SD	R^2
80	4.45	3.56	0.02	0.99	8.85	5.93	0.02	0.99
90	6.56	3.75	0.01	1.00	5.09	2.48	0.01	1.00
100	6.28	3.64	0.01	0.99	6.70	3.99	0.14	0.99

E- error, SD- standard deviation, RMSE -root mean square error

Meanwhile for the ID method, due to internal heating phenomenon, the saturated pressure becomes larger leading to a steeper slope of the MR

curves which show a non-linear pattern due to larger dependency of pressure on the chitosan concentration.

The optical, mechanical and barrier properties of these films are shown in Table 2.6. Film colour can be a factor in terms of consumer acceptance. OD films were more coloured than ID and AD films. L value indicates lightness, as the L value increases films are less coloured. Air-dried

Table 2.6. Mechanical and barrier properties of chitosan films prepared under different drying conditions

Properties		n	AD	OD	ID
Colour values	L	3	4.42±2.01	83.42±2.01	84.20±2.01
	a		-2.44±0.1	-3.20±0.2	-3.44±0.1
	b		7.14±0.14	11.40±0.15	8.16±0.18
Opacity (%)		3	1.36±0.25	1.72±0.32	1.42±0.16
Tensile strength (MPa)					
MD		5	59.38±4.48	52.71±4.27	52.34±3.73
TD		5	56.78±1.85	50±5.18	49.58±4.16
% Elongation					
MD		5	8.35±1.48	7.67±2.16	6.76±3.2
TD		5	6.24±2.4	6.42±3.26	5.40±4.2
Tearing strength (N)					
MD		5	0.016±0.01	0.016±0.018	0.012±0.006
TD		5	0.014±0.024	0.012±0.01	0.010±0.008
Burst strength (MPa)		5	3.13 ± 0.5	3.24 ± 0.5	2.94 ± 0.4
WVP(g.m/m^2 .kPa @ 90% RH at 38°C		4	0.020±0.002	0.01931 ± .001	0.0182 ±0.002
OTR (cc.m/m^2.day.kPa) 65% RH at 27°C		4	56±4	40±6	38±5
Crystallinity index (%)			25	43.31	38.5

n= number of replicates; AD= ambient drying; ID= Infrared drying; OD= Oven drying

films were more transparent than heat dried films. The 'a' value did not vary significantly indicating that the films became brown due to heating and hence variation in 'b' value (indicating yellowness) was significant between the three methods of drying. More yellowness was observed in OD films, due to the preferential drying of surface layers, and also it could be attributed to Maillard reaction products. Whereas in ID electromagnetic waves penetrate deep inside the solution and remove the moisture mass from inside out, and films were of less yellowness. Also the infrared rays provide some bleaching action as well. OD films had a higher opacity (1.72% ± 0.32) values followed by ID (1.42% ±0.16) and AD (1.36% ±0.25) films. AD film showed higher TS compared to heat dried films, which was attributed to excessive drying in the latter. The mean %ε of AD film was 8.35 ± 1.48 and 6.24 ± 2.4 in machine and transverse directions, respectively. These values were slightly more than those of heat dried films, and were comparable to earlier report (Kittur *et al.,* 1998), which had a value 7.1 and 6.2, but lesser than modified films, which had a value 27-33% (Caner *et al.,* 1998). The elongation of chitosan films was very less compared to synthetic plastic films, which will be in the range 250-300% (Brody and Marsh, 1997), which for some specific applications is a desirable attribute. The elongation characteristics of the films can however be improved by adding plasticizers (Caner *et al.,* 1998). Nevertheless, the tensile and elongation properties of chitosan films (49-52 MPa) were better than those of whey protein films where values were 23-49 MPa (Alcantara *et al.,* 1998) and were comparable to synthetic plastics. The tear strength values, which measure the tear propagation of the film showed no significant difference between these differentially dried films, although they were lesser than the earlier study, in the range of 1-2.95 N. (Kittur *et al.,* 1998).

The WVP of films prepared under different drying conditions are shown in Table 2.6. The heat-dried films showed lesser WVP (0.02 ± 0.002) compared to air-dried films (0.020 ± 0.002). The values were lower at lower water activity and higher at higher water activity (Kittur *et al.,* 1998), may be

due to hydrophilic nature of chitosan material. Higher WVP indicates poor barrier property of chitosan films. The WVP can be partially modified by adding plasticizers (Caner *et al.*, 1998).

The OTR values showed all the films to have superior barrier to oxygen transmission (Table 2.6). This property of chitosan film is of versatile use in modified atmosphere packaging of fruits and vegetables (Srinivasa *et al.,* 2003). The OTR values of chitosan films are far better than those of synthetic plastics such as LDPE and HDPE (Brody and Marsh, 1997), but were comparable to methylcellulose films (Donhowe and Fennema, 1993b). It is reported that the decrease in oxygen permeability is due to molecular orientation of the polymeric chains (Salame and Steingiser, 1977).

The X-ray diffraction patterns of chitosan films prepared under different drying conditions are shown in Fig.2.6. All the films showed

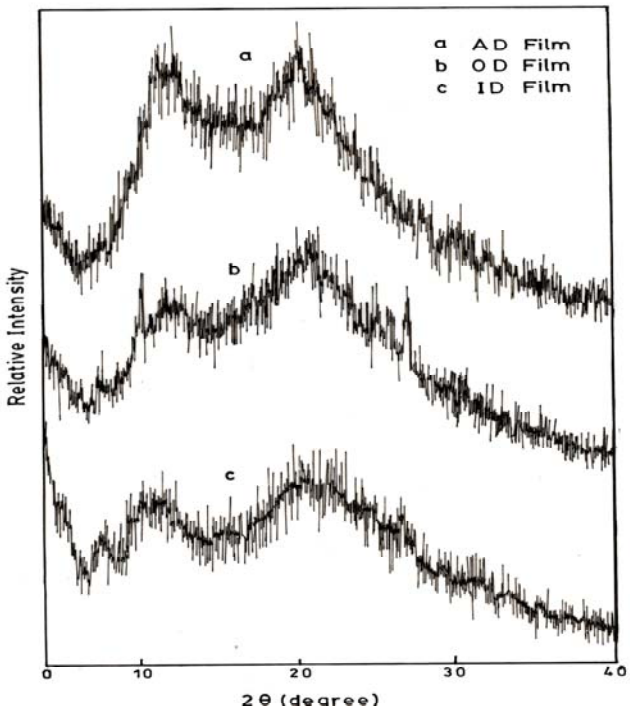


Fig. 2.6. X-ray diffraction pattern of chitosan films prepared under different drying conditions

strong reflections at 2θ around 10-12° and 20-22°. In addition, a small peak was observed at around 15° in OD films, which has been attributed to the anhydrous crystal lattice (Ogawa, 1991). The crystallinity index was more in ID films compared to OD films (Table 2.6). In an earlier report (Kato *et al.,*1978), elevated temperature was shown to enhance the crystallinity due to hydrophobic interactions. Increased crystallinity was also observed at higher temperatures in methylcellulose films (Donhowe and Fennema, 1993a)**.**

In an attempt to study microstructural changes in the films scanning electron microscopy (SEM) was used to visualize the surface topography of chitosan films prepared under different drying conditions. Results (data not shown) indicated no morphological changes, all the films showed smooth and uniform surface morphology without any cracks, voids and perforations.

Sorption and water vapor transmission rate of films at different RH

The relationship between a_w and moisture content (at constant temperature) is described by moisture isotherm. The moisture sorption of chitosan films was of sigmoid shape. The moisture content of films varied from 0.56 to 24.86 (%db) at 11% to 92% RH. The time to reach equilibrium was about 25-30 days for different films and at 92% RH, some mould was detected by visual inspection at edges and on the surface at the end of 25th day.

Moisture sorption isotherms of cellulose films and also for most of foodstuffs are basically sigmoid shape. A typical sorption curve of chitosan films is shown in Fig 2.7. At lower a_w the slope of the curve was less, (region A), which increases moderately at intermediate RH (region B). At high RH (region C) moisture content of chitosan film increases exponentially. Such large gain in moisture results in swelling. Swelling would cause conformational change in the microstructure of the film, that would not only increases moisture sorption, but also open up the polymer with an increase

in permeate flux. Water vapor acts as plasticizer inside the chitosan film matrix.

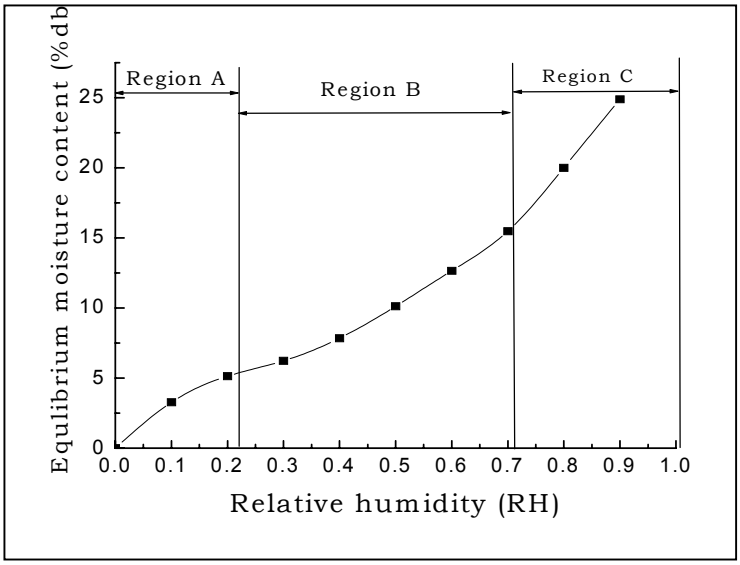


Fig. 2.7. The moisture sorption isotherm of chitosan films

The WVTR of the chitosan film at different RH are shown Fig. 2.8. The WVTR of the films ranged from 10 to 480 g/m^2 at 25 °C at 11 to 92 % RH.

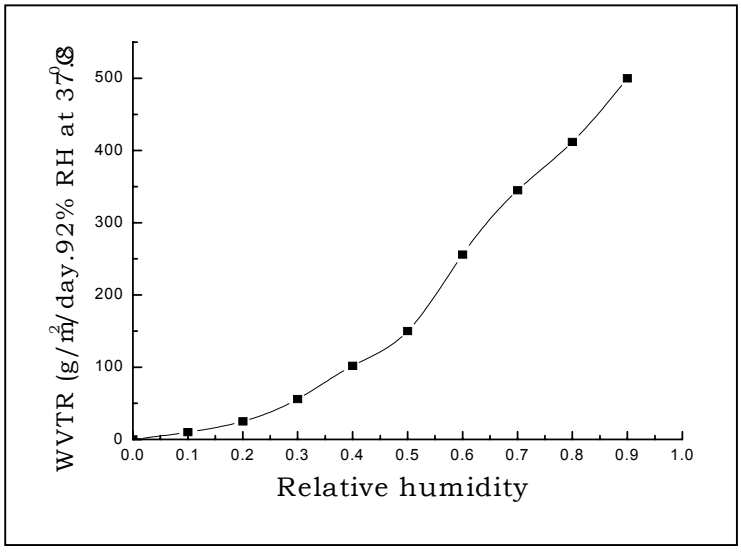


Fig. 2.8. WVTR of chitosan films under different RH

At higher RH, WVTR increases than steeply. At higher RH swelling result in deviation from Ficken behavior. This increased water vapor solubility leads to an increase in water vapor permeability. This suggest that more than polymer–penetrate interaction are occurring. Penetrate-penetrate interaction and formation of penetrate multiplayer that leads to mass flow of permeate result in large increase of WVTR.

Response surface methodology

Chitosan films had a slight yellow appearance, with the colour darkening as thickness of the film increased. As the water content of the films increased the films became soft. When submerged in water, the films became very soft and more elastic due to inconsistencies occurring in the film surface morphology, the film thickness ranged from 30 to 40 microns.

The effect of independent variable on TS, %ε and ME are shown in Table 2.7. The effect of independent variables on the response function of targeted parameters is represented by Analysis of variance (ANOVA) shown in Table 2.8 in coded levels of variables. The response surfaces generated for TS, %ε and ME are shown in figures 2.9-2.11 to aid in visualizing the effect of the variables.

Tensile strength

The TS varied between 5.5 to 40 M Pa, while %ε and ME varied between 5.17 to 39.9% and 400 to 939 MPa respectively. Excellent co-efficient of determination values of 0.998, 0.999 and 0.999 indicated the suitability of the fitted second order polynomials to predict the three response variables (TS, %ε and ME). The regression out put for the responses is shown in Table 2.8. In the process of optimization, the roots for TS ($\lambda 1$, $\lambda 2$, $\lambda 3$) of the auxiliary equation were –2.95, -8.825, -10.945 respectively indicating that the optimum condition is a case maximum.

Table 2.7. Experimentally determined dependent film properties

Independent variable (coded levels)			Dependent variables		
Temperature (°C)	Relative Humidity (%)	Storage period (days)	Tensile strength (MPa)	Elongation (%)	Modulus of elasticity (MPa)
-1	-1	0	27.15	13.50	938.92
1	-1	0	16.25	5.17	499.80
-1	1	0	35.91	24.56	400.00
1	1	0	20.70	4.65	869.33
-1	0	-1	19.02	10.97	465.00
1	0	-1	21.40	5.20	555.25
-1	0	1	30.60	19.90	681.40
1	0	1	5.50	1.30	682.20
0	-1	-1	31.30	29.84	858.33
0	1	-1	23.53	39.89	548.25
0	-1	1	24.50	34.53	835.04
0	1	1	29.16	22.97	624.75
0	0	0	40.00	23.20	623.22
0	0	0	38.70	22.25	624.75
0	0	0	38.00	22.91	623.22

Table 2.8. Analysis of variance (ANOVA) of storage conditions on mechanical parameters of film and their significance levels

Tensile strength	SS	MS	F
(1)TEMP (L)	280.80	280.80	272.62***
TEMP (Q)	442.31	442.31	429.43***
(2)RH (L)	27.17	27.17	26.38*
RH (Q)	32.19	32.19	31.25 *
(3)DAYS (L)	4.81	4.81	4.67 NS
DAYS (Q)	287.56	287.56	279.18 ***
1L by 2L	4.64	4.64	4.51 NS
1L by 2Q	1.44	1.44	1.39 NS
1Q by 2L	33.29	33.29	32.32 *
1L by 3L	188.79	188.79	183.29***
1Q by 3L	1.24	1.24	1.20 NS
2L by 3L	38.63	38.63	37.50 *
R^2=0.998			
Elongation (%)			
(1)TEMP (L)	326.84	326.84	1378.86***
TEMP (Q)	1022.46	1022.46	4313.59***
(2)RH (L)	19.15	19.15	80.79*
RH (Q)	125.25	125.25	528.39 ***
(3)DAYS (L)	0.24	0.24	0.99 NS
DAYS (Q)	37.73	37.73	159.18***
1L by 2L	33.52	33.52	141.43 ***
1L by 2Q	1.87	1.87	7.90 NS
1Q by 2L	18.15	18.15	76.57 *
1L by 3L	41.15	41.15	173.61 ***
1Q by 3L	37.24	37.24	157.10 ***
2L by 3L	116.75	116.75	492.54 ***
R^2=0.999			
MODULUS			
(1)TEMP (L)	1147.16	1147.16	1470.15 ***
TEMP (Q)	4186.79	4186.79	5365.61 ***
(2)RH (L)	36906.94	36906.94	47298.39 ***
RH (Q)	27918.98	27918.98	35779.80***
(3)DAYS (L)	27373.34	27373.34	35080.53***
DAYS (Q)	128.80	128.80	165.07 ***
1L by 2L	206320.35	206320.35	264411.57***
1L by 2Q	462.69	462.69	592.96 ***
1Q by 2L	15398.37	15398.37	19733.91***
1L by 3L	2000.33	2000.33	2563.53***
1Q by 3L	10522.65	10522.65	13485.39***
2L by 3L	2489.51	2489.51	3190.45***
R^2=0.999			

* 5% significance, ** 1% significance, *** 0.1% significance, NS Not significance

Table 2.9. Regression co-efficient of dependent film properties and their significance

Response parameters	Tensile strength	Percent elongation	Modulus of elasticity
Mean/Interc.	-35.49 NS	15.19 NS	2215.54***
(1)TEMP(L)	2.37*	3.52***	12.48***
TEMP(Q)	-0.04 *	-0.06***	-0.84***
(2)RH (L)	0.58 NS	-1.19*	-47.73***
RH (Q)	-0.00 NS	0.02***	0.26***
(3)DAYS(L)	10.89***	3.36*	13.68***
DAYS(Q)	-0.98***	0.36***	0.66***
1L by 2L	-0.01 NS	-0.01 NS	0.24***
1L by 2Q	-0.00 NS	-0.00 NS	-0.00 NS
1Q by 2L	0.00*	0.00 NS	0.01 NS
1L by 3L	-0.07 NS	-0.19***	-2.79***
1Q by 3L	-0.00 NS	0.00 NS	0.05 NS
2L by 3L	0.05*	-0.09 NS	0.42***

* 5% significance, ** 1% significance, *** 0.1% significance, NS Not significance

Fig.2.9a shows variation of TS with respect to RH and temperature, keeping the storage period constant. It was observed that with increase in temperature TS reaches maximum at 30 °C and then decreased. At low temperature and higher RH the TS was more compared to that at lower RH. Fig.2.9 b shows the effect of storage period and RH when temperature is kept constant. TS reaches a maximum at around 60% RH and storage period of around 5.5 days. Fig 2.9 c shows the variation of TS with temperature and storage period keeping the RH constant. TS reached a maximum of 44.42 MPa around a temperature of 29 °C and storage period of 5.5 days

The effect of temperature, RH and storage period on TS of the films is shown as surface graphs and depicted in Figs. 2.9 a-c.

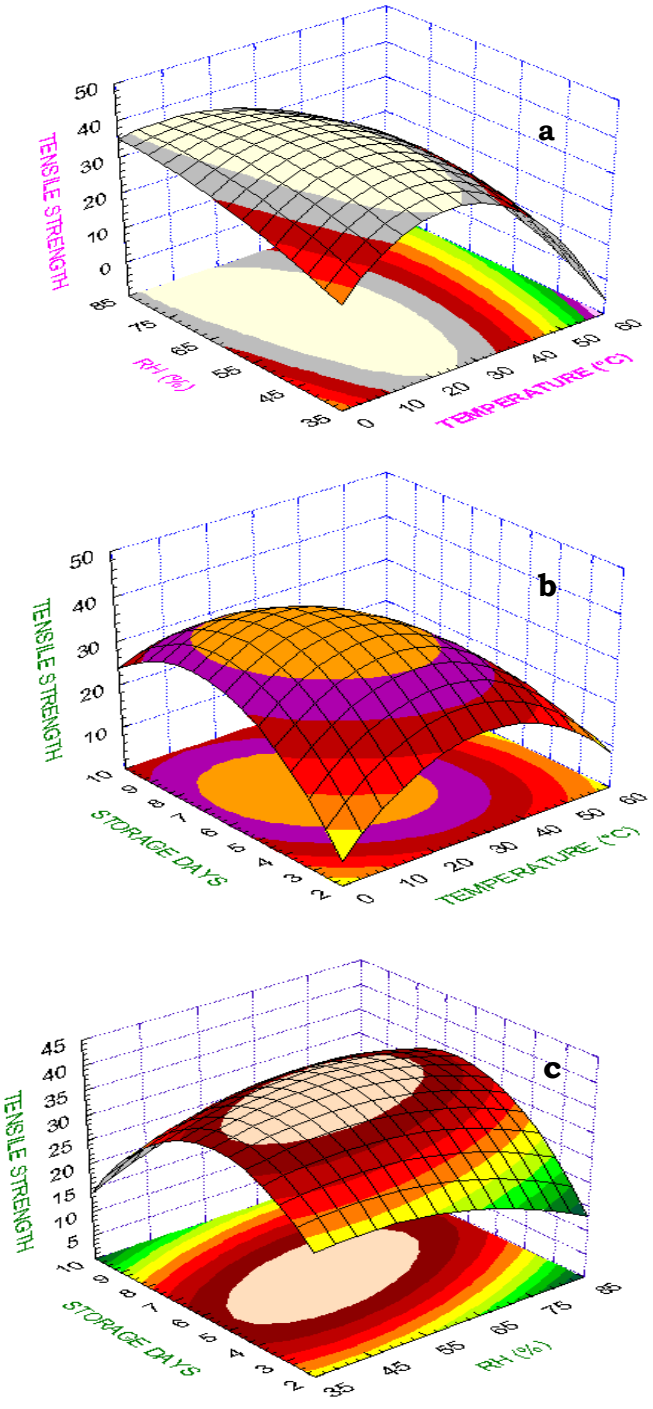


Fig. 2.9. Response surface of tensile strength with independent variables **a)** RH v/s temperature, **b)** Days v/s RH and **c)** Days v/s temperature.

Elongation

The elongation of the film is an indicator of extensibility of the films. The ANOVA results indicated that temperature and RH played a vital role in

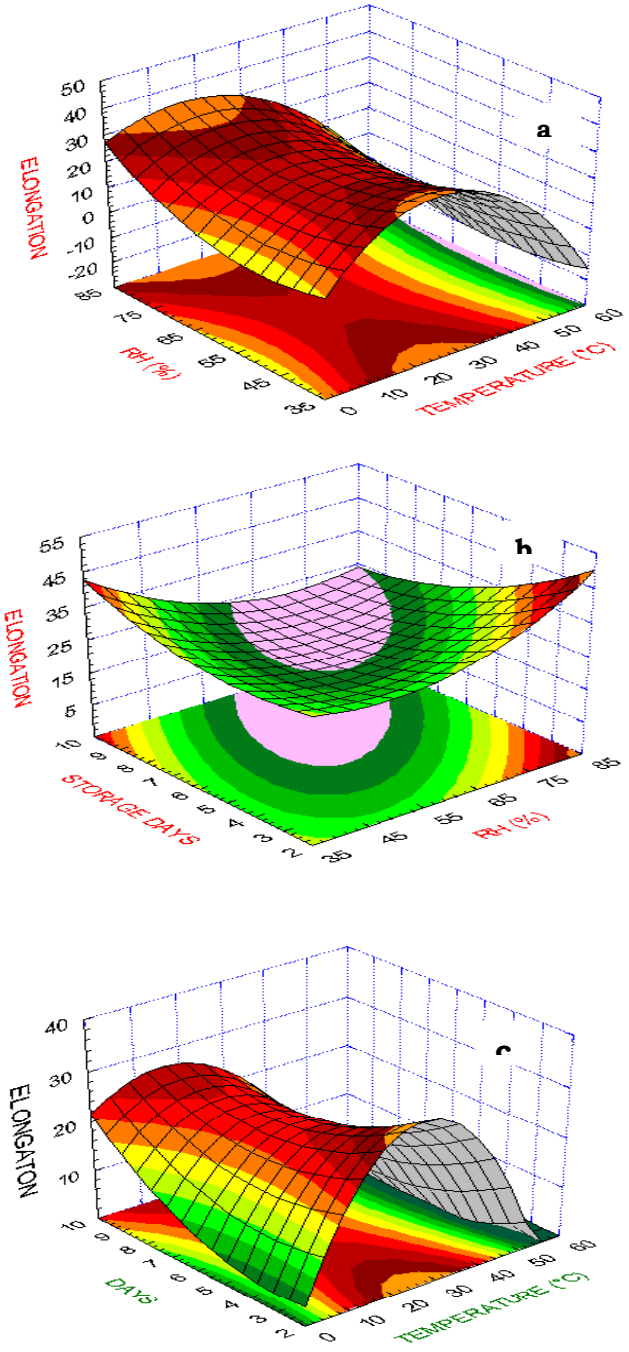


Fig. 2.10. Response surface of % elongation with independent variables **a)** RH v/s temperature, **b)** Days v/s RH and **c)** Days v/s temperature

determining the elongation properties of the films. Highly significant values were obtained for quadratic effect as well as linear effect of variables.(Table 2.8). The influence of RH factor can be explained by its plasticizing effect, as water loosens the interaction with chain links in the film. Canonical results indicated that the nature of the optimum condition for elongation was a saddle point. The roots were 5.82, 3.19, and -16.64 for λ1, λ2, and λ3, respectively. Fig.2.10a shows variation of the elongation with respect to RH and temperature, keeping the storage period constant. It was observed that with increases in temperature elongation increased, reaches a maximum when the was around 30-40 °C and then decreased. At low temperature and higher RH the elongation was more, compared to that at lower temperature and LOW RH. Fig.2.10b shows the effect of storage period and RH when temperature as kept constant. Elongation showed an increase during storage as the RH increased. Fig 2.10c shows the variation of elongation with temperature and storage period keeping the RH constant. The % elongation reached a maximum at around 30 °C, and then decreased.

Modulus of elasticity

ME is also an indicator of the stiffness of the film. Fig. 2.11 illustrates the three dimensional response surfaces for ME. The values were least at high RH and low temperature and increased with temperature and decrease with RH when storage period was kept constant (Fig. 2.11a). At lower temperatures, the ME of film decreases with increasing RH drastically.

Simultaneous optimization of parameters observed with a overall desirability score of 0.8429, when TS, %ε and ME value were 35.79 MPa, 19.86% and 896.73 MPa, respectively (Table 2.9). These values were obtained when the independent variables i.e., temperature, RH and storage days were 20.1°C, 40% and ~7, days respectively. The experiment was repeated at actual levels of variable and found it is significant (p =0.05).

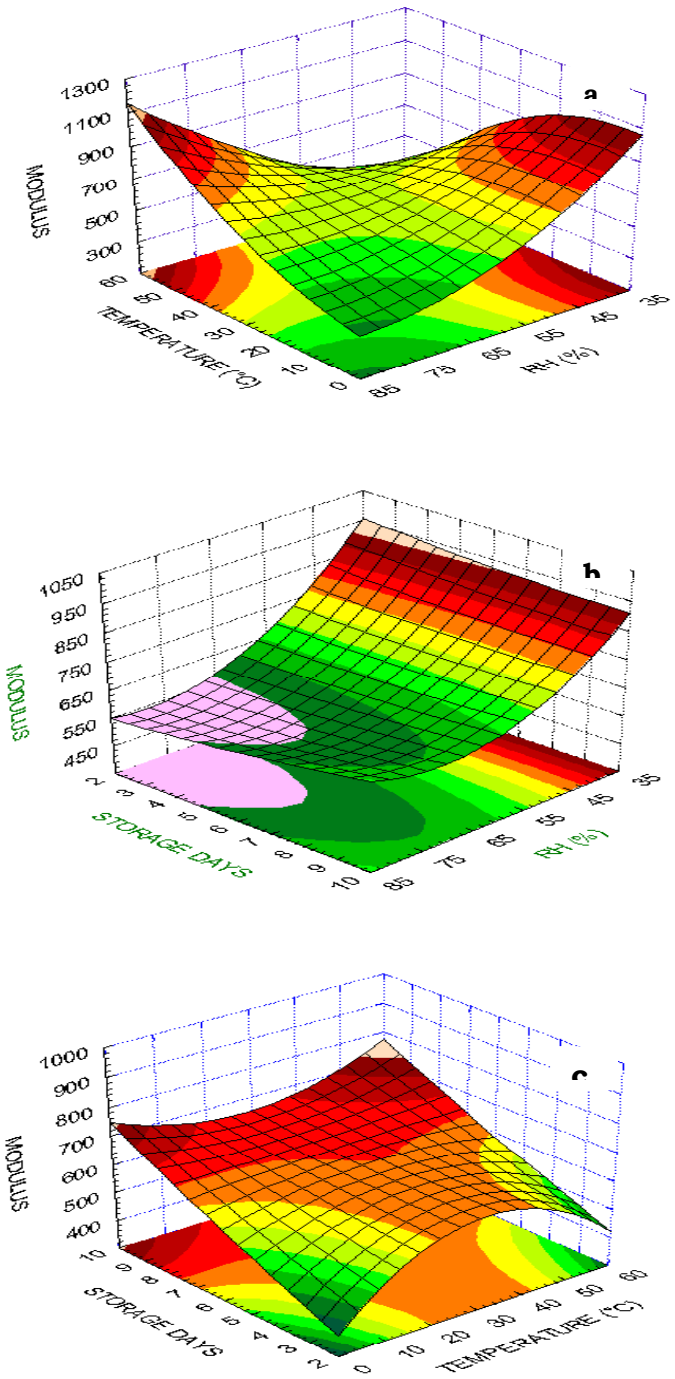


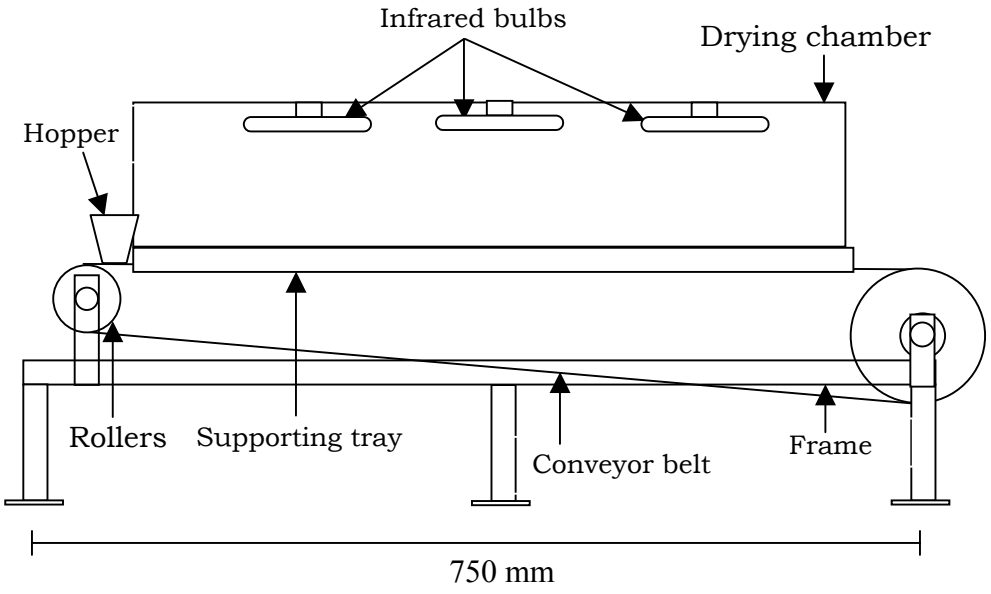
Fig. 2.11. Response surface of Modulus of elasticity with independent variables **a**) RH v/s temperature, **b**) Days v/s RH and **c**) Days v/s temperature

Table 2.9. Simultaneous optimization parameters and the overall desirability

Independent storage condition	Actual levels of variables	Tensile	Elongation	Modulus	Overall desirability
Temp. (°C)	20.1				
RH (%)	40	35.79	29.86	896.73	0.8429
Storage period [Days]	~7				

Wet casting unit

The earlier result of chitosan films preparation showed infrared drying is faster compared to other methods of drying and no significant differences were observed in their properties (Table 2.6). With these in background an attempt was made to fabricate a prototype model for continuous production of chitosan film under IR drying. The model design, shown in Fig.2.12, consists of two rollers over which the conveyor belt is moved. On the top, the belt is supported by tray to prevent any sagging and to maintain level. The drying chamber is made up of stainless steel frame to with three IR bulbs (500 watts) are fixed at the top. The bulb is covered with reflectors for uniform heat distribution over the belt. The hopper is fixed at the side of the drying chamber to spread the solution uniformly over the belt. The whole unit is mounted on the cast iron frame and kept levelled by operating leveling screws. The belt is moved by means of a motor, which is connected to roller, the speed of which is controlled by electronic circuits. Provision is also made for manual movement of the belt by means of handle. The fabricated model is shown in Fig. 2.13.



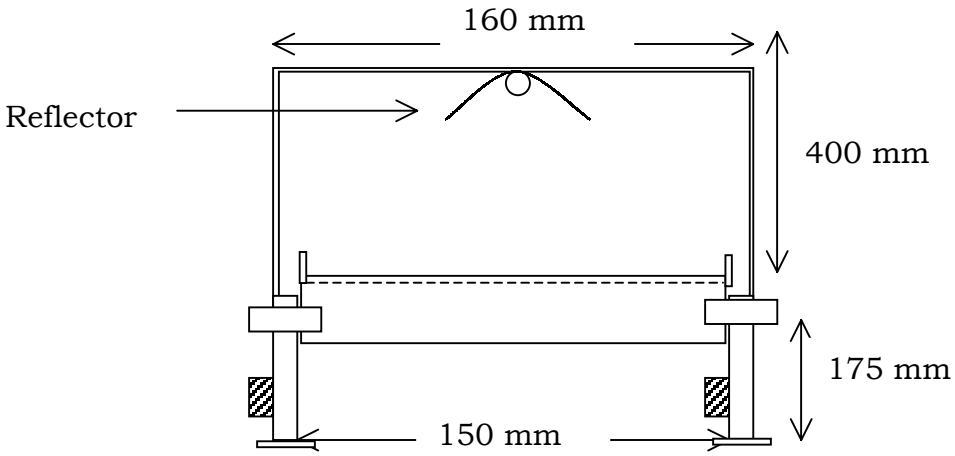


Fig.2.12. Design of continuous wet casting machine prototype unit

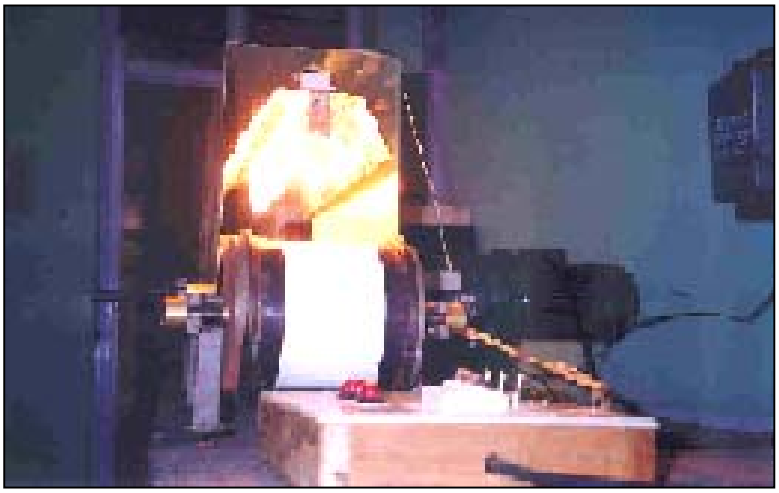


Fig.2.13. Prototype model of continuous wet casting unit

Chitosan solution (1% in 0.5% acetic acid) was prepared and spread uniformly over the belt through hopper. The belt was moved with a speed of 1.0 cm/min. In the drying chamber the temperature was maintained at 100 °C by. When the dried films came out of the chamber, they were peeled off., from the belt. In the present study the width was maintained about 75-80mm, which could be varied by using appropriate prototype design. The parameters essential for continuous of film preparation are viscosity of chitosan solution, drying temperature, cooling chamber and a few others such as maintaining precise RH, steam treatment to minimize acidic note,

etc). The properties of the films are modified by adding plasticizers or colours for different packaging applications.

Conclusions

Chitosan films were prepared using acetic acid and polyester as the base material. Infrared drying was found to be faster compared to oven drying and ambient drying conditions. Thin layer model was used to study the drying curves and to determine kinetic constants. Chitosan films did not show much difference in the properties under different drying conditions. The sorption isotherm of chitosan film showed typical sigmoid shape. Simultaneous optimization by desirability approach resulted in an overall desirability score of 0.8429, where TS, %ε and ME values were 35.79 MPa, 19.86% and 896.73 MPa respectively and these values were obtained when the independent variables such as temperature, RH and storage were 20.1°C, 40% and ~ 7 days, respectively. Fabrication of a prototype wet casting unit for continuous production of chitosan film was also attempted.

Introduction

Polymer blending is one of the effective methods for providing new desirable polymeric materials for a variety of applications. Plasticizing agent is an important ingredient generally used to overcome the brittleness of the biopolymeric films. Brittleness is an inherent quality attributed to the complex/branched primary structure and weak intermolecular forces of natural biopolymers. Plasticizers soften the rigidity of the film structure, increase the mobility of the biopolymeric chains and reduce the intermolecular forces, thus improving the mechanical properties (elongation). Various workers have reported on the utilization of glycerol as plasticizer to produce light yellow, transparent protein films (Cunningham *et al.*, 2000). Glycerol is shown to improve film flexibility, reduce film puncture strength, elasticity and water vapour barrier properties of wheat gluten films (Gontard *et al.*, 1993). Alginate films containing 50% or more of sodium lactate had elongation in excess of 13%. Use of sorbitol exhibited best water vapor permeability values (Parries *et al.*, 1995). Arvanitoyannis *et al.* (1996) revealed that films made out of sodium casienate and soluble starch contained sugar and glycerol as plasticizers. Polyethylene glycol (PEG) has been used in methylcellulose-based films (Turhan *et al.*, 2001).

As for as chitosan films and chitosan-modified films are concerned, use of PEG (0.25 and 0.5%) resulted in reduced tensile strength and WVTR of the film but %ε increased (Wiles *et al.*, 2000). Butler *et al.* (1996) reported modified chitosan films containing glycerol (0.25 and 0.5%), to show decrease in (TS), increase in %ε, but oxygen and ethylene permeability remained constant during the storage period. Qurashi *et al.* (1992) reported decrease in TS and %ε with increase in the amount of PVP in the chitosan blend films, which were colourless and transparent, and did not show any microbial or fungal growth upon storage. Blair *et al.* (1987) modified the chitosan film by blending with polyvinyl alcohol (PVA) and studied the TS and %ε. Nakajima *et al.* (1980) reported chitosan sample with high amino

group content and long chain length showed lower moisture regime than sample with low amino group and shorter chain length and the permeability of films decreased as the degree of deacetylation (DD) increased. Blair *et al.* (1987) reported the mechanical properties of chitosan-PVA blend films. Park *et al.* (2001) studied the properties of chitosan–PVA blend films prepared using different solvents and observed the blend films to have better properties when acetic acid was used as a solvent. So for, very limited work is done on modifying the chitosan films by fatty acids.

An important role of a packaging film or an edible film is to reduce exchange of water between the product and environment (Coupland *et al.* 2000). The barrier property of such films depends on both molecular diffusion co-efficient and solubility of water in the matrix (McHugh and Krochta, 1994). The moisture sorption isotherm is a means to characterize the water absorption property of the film, which in turn is transmitted to the product inside. Knowledge of sorption isotherm is also important for predicting stability and quality changes during packaging and storage of food products. Chirife and Iglesias (1978) reviewed a number of isotherm equations for food and food products, but relatively only a few isotherms are reported for packaging film materials. Gennadios and Weller (1994) reported isotherm for corn zein, wheat gluten and mixed protein films and showed GAB isotherm model to have good description of their data. Chinnan and Park (1995) reported sorption isotherm for methyl and hydroxypropyl cellulose films.

In this chapter, the preparation of chitosan films blended with ploysols (glycerol, sorbitol and PEG), fatty acids (stearic and palmitic acids) and a synthetic water-soluble polymer (PVA) and a study of their optical, mechanical and barrier properties are reported. Also results of X-ray, DSC, sorption and biodegradation studies are included.

Materials and Methods

Chitosan of M_W (2,00,000,CH2) was used to blend with polyols, fatty acids and water-soluble polymer. Plasticizers glycerol, sorbitol and polyethylene glycol (PEG) were procured from Sisco Research Lab, Bombay; stearic and palmitic acids procured from Merck India, and PVA was from Sd Fine Chemicals, Mumbai, India.

Chitosan blend films were prepared using different concentrations of plasticizers, which were optimized to 0.4% for glycerol, 0.5% for sorbitol, and 0.6% for PEG and 0.5% for fatty acid. At higher concentration of plasticizers difficulty was experienced in peeling off the films. Chitosan blend films were prepared by adding different concentration of plasticizer to the chitosan solution (1% w/v) and stirred for 60 min. For fatty acid blend films, chitosan solution was heated to 60^0 C and fatty acid was added and later manually stirred using a glass rod. Chitosan-PVA blend films were prepared by dissolving PVA in hot water with constant stirring, after its complete solubilization, the solution was cooled, and 0.5% acetic acid along with chitosan were added and stirred. The chitosan (1%, w/v) and PVA (2.3%, w/v) concentrations were optimized for the preparation of pure and blend chitosan-PVA films. Blend films were prepared as mentioned before. The various properties of blend films were established in Chapter II.

Sorption isotherm models

A number of sorption isotherm models have been reported in the literature. In the present study BET (Labuza, 1968), GAB (Bizot 1984), Caurie (Caurie, 1970), Halsey, Smith, Oswin, Bradley (Chirife and Iglesias, 1978), and Harkins- Jura (Labuza, 1968) models were used for fitting the sorption data. The equations were rearranged to linear form to determine the

appropriate constants (Table 3.1) by regression analysis using MS-Excel Software (Microsoft Inc, 2000).

Table 3.1 Linerized form sorption models.

Sl. No	Model	Equation	Linearized equation	Constants
1	BET	$M = \frac{M_m C a_w}{(1-a_w)[(1+(C-1)a_w]}$	$\frac{a_w}{(1-a_w)M} = \frac{(C-1)}{M_m * C} + \frac{1}{M_m * C}$	M_m, C
2	GAB	$\frac{M}{M_0} = \frac{GKa_w}{(1-Ka_w)[(1-Ka_w+GKa_w]}$	$M = \frac{a_w}{(A * a_w^2) + B * a_w + C]}$ Where $A = \frac{K}{M_0[(1/G)-1]}$ $B = \frac{1}{M_0[1-(2/G)]}$ $C = \frac{1}{M_0 GK}$	M_0, G, K
3	Caurie	$\ln M = a + b * a_w$	$\ln M = a + b * a_w$	a, b
4	Halsey	$\ln = \exp\left[\frac{-a}{RT\theta^r}\right]$	$\ln(\theta) = a + r[\ln - (\ln a_w)]$	a, r
5	Smith	$M = M_b - M_a * \ln(a_w)$	$M = M_b - M_a * \ln(1-a_w)$	M_a, M_b
6	Oswin	$M = a\left[\frac{a_w}{(1-a_w)}\right]^n$	$ln\, M = ln\, a + n * ln\left[\frac{a_w}{(1-a_w)}\right]$	a, n
7	Bradley	$\ln n\left[\frac{1}{a_w}\right] = K_1 * K_2{}^M$	$ln(ln\left[\frac{1}{a_w}\right] = lnK_1 + M * ln(K_2)$	K_1, K_2
8	Harkins -Jura	$ln(a_w) = B - \left[\frac{A}{M^2}\right]$	$\ln(\ln a_w) = \ln A + B * \ln(M^2)$	A, B

The sorption data were analyzed according to the models and the corresponding constants were determined. The goodness of fit of each model was computed in terms of coefficient of determination (R^2) from the plot of experimental (M_{exp}) and predicted (M_{pre}) sorption moisture and root mean square error (RMSE) values, as follows,

$$RMSE = \sqrt{\left[\frac{\sum (M_{exp} - M_{Prd})^2}{N}\right]} \qquad \text{-------- (1)}$$

Where, M_{exp} = Moisture content experimental (% db),

M_{prd} = Moisture content predicted (% db),

N = number of observations

Biodegradation of chitosan based films

Native and blend films of size (3 x 3 cm) were exposed to compost/ mud mixture by completely burying well inside, along with control film sample. The moisture content in the compost/ mud was maintained at 25% throughout the study. The samples were removed at frequent intervals to observe any biodegradation, visibly and also by scanning electron microscopy (SEM).

Results and Discussion

For clarity in understanding this part is divided into three sections, namely A. Polyols, B. Fatty acids, and C. Synthetic water soluble polymer.

A. Polyols

During wet casting under IR drying, the film obtained was found to be affected by the nature of solution viscosity. No significant differences were observed in viscosity of the chitosan solution with the addition of glycerol and sorbitol, whereas PEG blended chitosan solution showed a decrease in

viscosity by ~100 cps. The density of the films decreased as plasticizer concentration increased (Fig.3.1). The native chitosan films showed a density value of 1.4067 g/ml. Addition of glycerol into chitosan-glycerol blend films did not show much variation in the density value compared to native film, except for slight decrease to 1.3915 g/ml at 0.4% of optimized concentration. A similar trend was observed in sorbitol blend films where density decreased to 1.3215 g/ml, but maximum decrease in density value was observed in PEG-blend films, with a value of 1.2930 g/ml. The glycerol films showed a very low change in density compared to other plasticizers, whereas in PEG blend films the density decreased drastically, may be due to its long chain/ molecular weight.

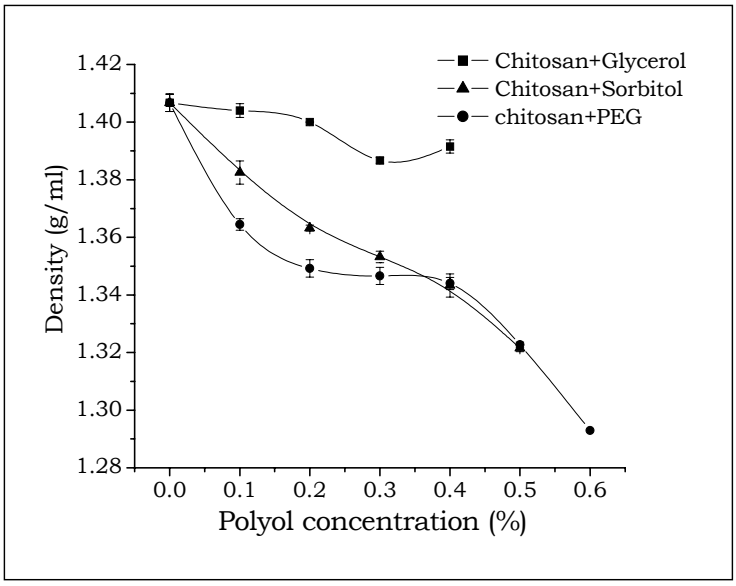


Fig 3.1. Density of Chitosan-Polyol blend films

In colour values, the blend films showed no significant change in lightness (L) value. The yellowness of the film increased with the addition of plasticizers as indicated by a and b values (Table 3.2), may be due to oxidative browning reaction when kept for long storage period. The chitosan-glycerol blend films have more tendencies for the above process. A similar result was observed when glycerol was added to proteins to produce films. Opacity, indication of the transparency of the films, showed no significant

difference in opacity values when glycerol and sorbitol were added to the films (Table 3.2), but least was observed with chitosan-glycerol blend films. The opacity of film increased with the addition of PEG, at optimized concentration (0.6%) the value was 14.48%. The addition of PEG resulted in increased thickness of the film, probably due to the formation of a layer of PEG over the preformed films, without forming a interlinking chain with chitosan molecules, even at higher concentration of PEG. The water

Table 3.2. Colour, Opacity and WRV of chitosan films modified with polyols

Glycerol	Conc. (%)	L	a	b	Opacity	WRV
	0.0	95.84 ± 1.48	-1.20 ± 0.23	8.26 ±1.23	8.11 ± 0.23	6.08 ± 0.03
	0.1	96.25 ± 0.04	-0.84 ± 0.02	2.01 ± 0.07	8.25 ± 0.31	1.90 ± 0.35
	0.2	95.65 ± 0.75	-2.19 ± 0.54	5.76 ± 1.36	7.23 ± 0.26	1.98 ± 0.23
	0.3	96.76 ± 0.56	-0.99 ± 0.32	2.04 ± 1.48	8.09 ± 0.16	1.80 ± 0.24
	0.4	95.78 ± 0.48	-1.52 ± 0.45	4.06 ± 0.81	8.21 ± 0.24	1.93 ± 0.27

Sorbitol	Conc. (%)	L	a	b	Opacity	WRV
	0.0	95.84 ± 1.48	-1.20 ± 0.23	8.26 ± 1.23	8.11 ± 0.23	6.08 ± 0.03
	0.1	95.89 ± 1.07	-1.52 ± 0.16	3.48 ± 0.18	8.39 ± 0.81	4.01 ± 0.15
	0.2	95.76 ± 0.35	-1.79 ± 0.43	4.76 ± 1.41	8.48 ± 0.23	3.32 ± 0.75
	0.3	93.74 ± 1.56	-1.93 ± 0.22	3.59 ± 0.24	9.06 ± 0.19	2.42 ± 0.38
	0.4	95.49 ± 012	-1.78 ± 0.34	4.89 ± 0.37	8.15 ± 0.46	2.01 ± 0.54
	0.5	95.49 ± 0.28	-2.08 ± 0.14	5.89 ± 0.46	8.28 ± 0.66	1.70 ± 0.32

PEG	Conc. (%)	L	a	b	Opacity	WRV
	0.0	95.84 ± 1.48	-1.20 ± 0.23	8.26 ± 1.23	8.11 ± 0.23	6.08 ± 0.03
	0.1	95.48 ± 0.47	-1.40 ± 0.26	2.12 ± 0.12	8.39 ± 0.81	1.78 ± 0.28
	0.2	96.42 ± 1.87	-1.60 ± 0.31	3.81 ± 1.43	8.78 ± 0.33	2.56 ± 0.34
	0.3	93.74 ± 1.56	-1.93 ± 0.22	3.59 ± 0.24	9.06 ± 0.19	3.36 ± 0.61
	0.4	89.43 ± 0.25	-1.65 ± 0.34	3.34 ± 0.02	13.29 ± 2.06	3.84 ± 0.21
	0.5	88.32 ± 0.38	-1.88 ± 0.23	3.65 ± 0.12	14.23 ± 1.28	4.16 ± 0.19
	0.6	89.23 ± 0.02	-1.38 ± 0.03	2.60 ± 0.03	14.48 ± 1.86	5.36 ± 0.45

retention values (WRV) of blend films are shown in Table 3.2. A drastic decrease in WRV was observed in glycerol blend films, where with addition of 0.1% of glycerol, the WRV was 1.90, with further addition no considerable differences were observed. A gradual decreasing trend was observed in sorbitol blend films, where the value decreased to 1.70 at optimized concentration (0.5%). The addition of PEG (0.1%) to the blend films showed decrease in WRV, with further addition of PEG the films uptake the water and at 0.6% WRV was found to be 5.36.

Mechanical properties

The tensile strength (TS), percentage elongation (%ε) and modulus of elasticity (ME) could be used to describe how the mechanical properties are related to their chemical structure. Tensile strength indicates the maximum tensile stress that the film can sustain. Elongation is the maximum change in length of a test specimen before breaking. Modulus of elasticity is a measure of the stiffness of the film. The results indicated that these properties vary with the nature of plasticizers used. Mechanical properties of the films were studied in both directions to know orientation of the molecule. The TS value of glycerol blend chitosan films showed a decreasing trend in both the directions with addition of glycerol (Fig.3.2). The film looses 50% of strength at 0.3% and 70% at 0.4% of glycerol. Sorbitol blend films also showed a decreasing trend in TS, which was apparent over and above 0.3% sorbitol; at optimized concentration its TS was 11.26 MPa. However, PEG blend films behaved differently compared to other plasticizers. The TS of films showed initial decrease and later an increasing trend. With the addition of 0.1% and 0.2% PEG the TS decreased to 32.71 and 28.95 MPa, whereas at 0.3% PEG no significant differences were observed (28.42 MPa). At optimized concentration the TS was found to be 38.15 MPa, which was comparable to that of native chitosan film.

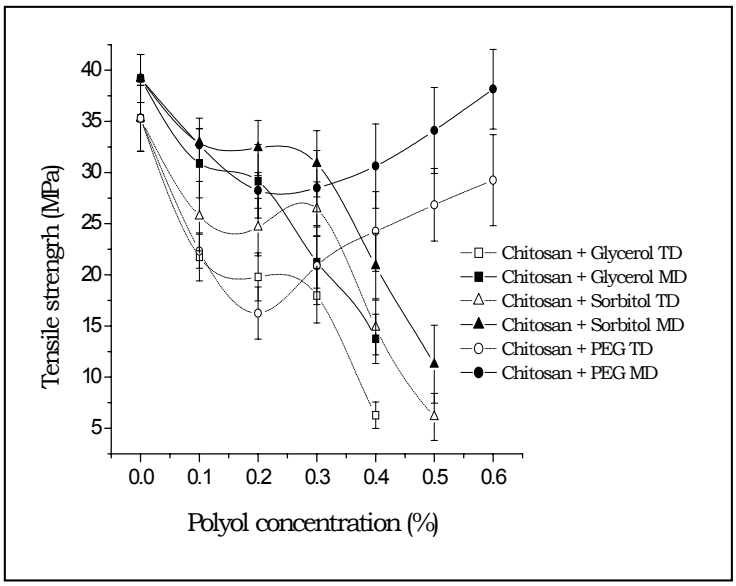


Fig 3.2. Tensile strength of Chitosan-Polyol blend films

The TS and %ε are inversely correlated. The %ε values showed an increasing trend with the addition of plasticizers (Fig. 3.3). Glycerol film showed maximum %ε values (42.5%) at 0.3% concentration and later decreased with further addition. Sorbitol and PEG blend films showed maximum elongation at optimized levels. Glycerol was miscible easily with chitosan and became more flexible compared to other plasticizers.

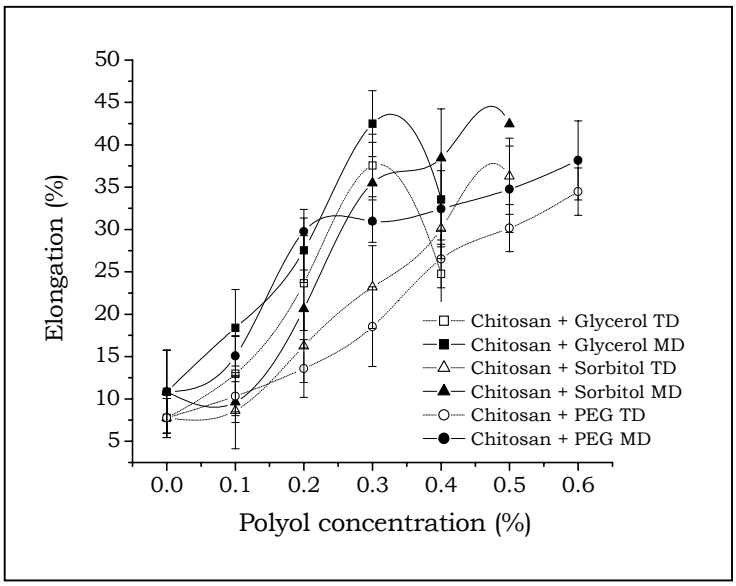


Fig. 3.3. % Elongation of Chitosan-Polyol blend films

Modulus of elasticity (ME) decreased with the addition of plasticizer (Fig.3.4). The glycerol and sorbitol blend films showed a drastic decrease in ME with small addition. In glycerol blend film with 0.1% addition the ME decreased by 50%, whereas in sorbitol and PEG, the decrease was 25% and

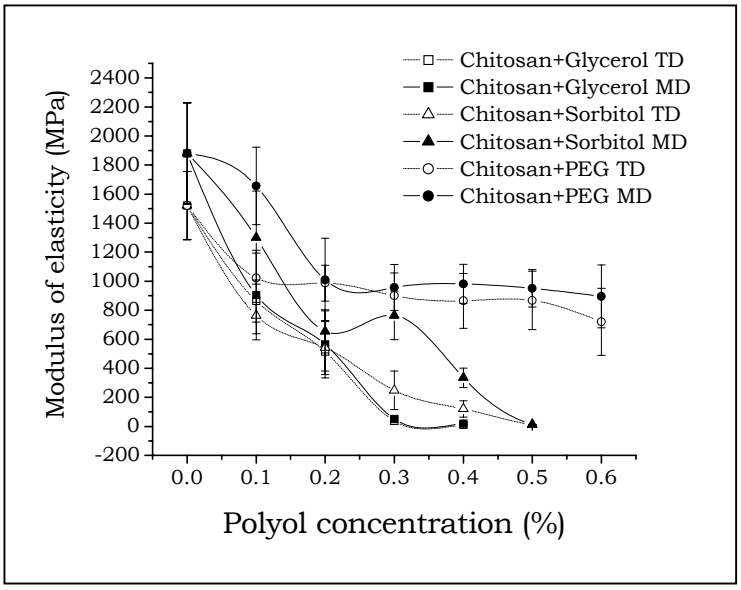


Fig. 3.4. Modulus of elasticity of Chitosan-Polyol blend films

10%, respectively. At optimized concentration the ME values were 19.35, 13.14 and 895 MPa, respectively for glycerol, sorbitol, and PEG blend films. This shows PEG blend films to be much stiffer. This may partly be due to the introduction of glycerol or sorbitol moieties resulting in drastic chain flexibility, thereby the rigidity of native chitosan disappears, whereas with PEG, due to its higher molecular weight it forms a separate molecular layer over the chitosan film and retains stiffness. Also the smaller size of glycerol influences the mechanical property of the film, giving more elongation to the film compared to other plasticizers. Arvanitoyannis *et al.* (1996) reported that blending of gelatin with chitosan in addition to glycerol and sorbitol, gave films whose TS and ME were decreased to 130-83 and 2050-1890 MPa, respectively. While Caner *et al.* (1998) reported a decrease from 32 MPa to 17 MPa with the addition of PEG, while % elongation increased to 42 %. The

TS and %ε of methylcellulose film were decreased and increased with addition of different plasticizers (Donhowe and Fennema, 1993a).

Tear strength values indicate the force required for tear propagation. With the addition of plasticizer no considerable difference in tear values were observed (Fig.3.5). The highest value was found in 0.2 % glycerol blend film, whereas sorbitol and PEG blend films showed a lower value of 0.03 and 0.03, respectively at optimized concentration levels. Fig.3.6. shows the impact strength of the modified films. All the films showed initial increase in impact strength with the addition of polyols, except for PEG blend films, which showed a higher value. Sorbitol blend films at and above 0.3%

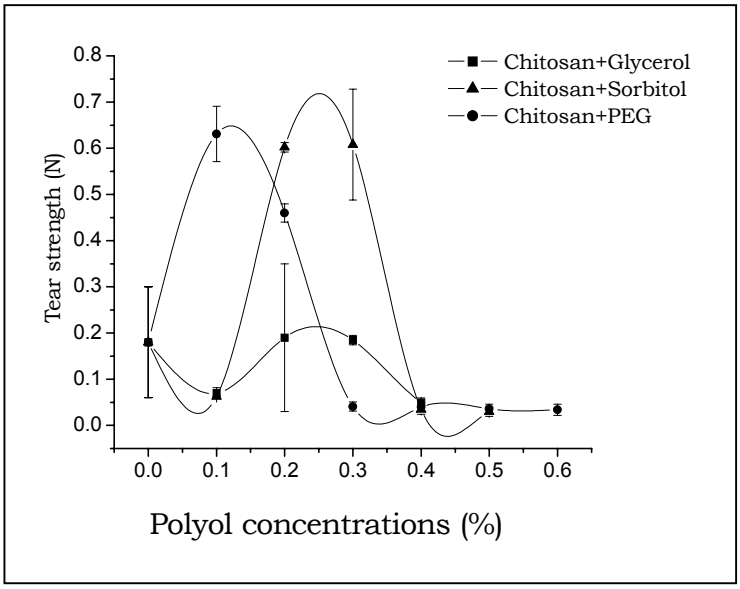


Fig. 3.5. Tearing strength of Chitosan-Polyol blend films

concentration showed a decrease in impact strength. Burst strength and impact strength measure the sudden load resisted by the packaging materials. No reports on the impact strength of the biopolymer films compared to synthetic polymer films are available in the literature. Fig.3.7 displays the effect of polyol content on the average burst strength of chitosan blend films. The average values ranged from 102-89 kPa for glycerol,

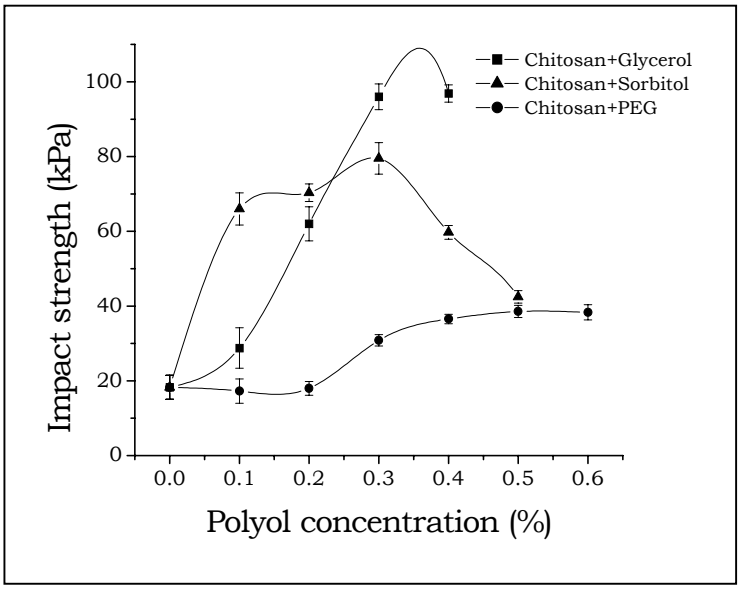


Fig. 3.6. Impact strength of Chitosan-Polyol blend films

102-90 kPa for sorbitol, and 102- 189 kPa for PEG blend chitosan films at their optimized levels. Fig. 3.7. showed a decreasing trend with the addition of glycerol and sorbitol, whereas addition of PEG showed an increasing trend.

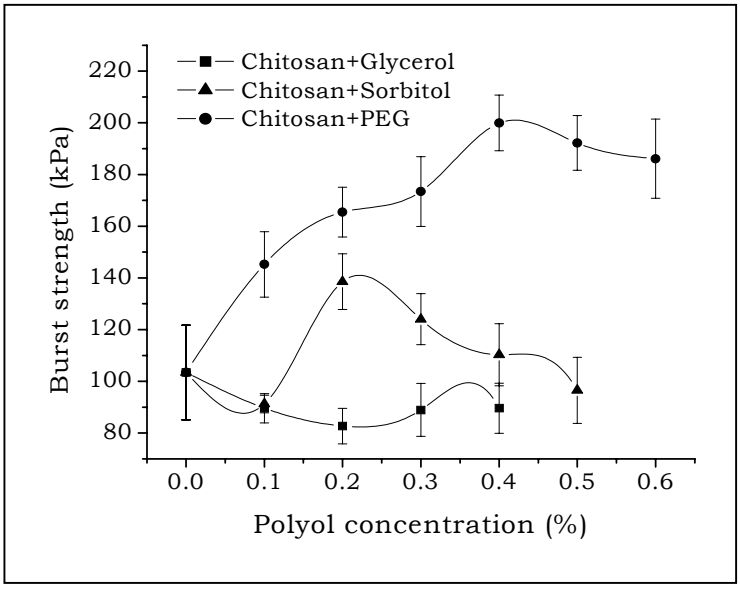


Fig. 3.7. Burst strength of Chitosan-Polyol blend films

WVP of native chitosan film was 0.01322 g.m/m^2.day.kPa. Incorporation of plasticizers affects the WVP of chitosan films (Fig. 3.8) to considerable extent. WVP of chitosan-glycerol blend films decreased with the addition of glycerol, at optimized concentration (0.4%) the value was found to be 0.008 g.m/m^2.day.kPa. This decrease could be due to the formation of hydrogen bonding in between the crevices of chitosan chains, thereby influencing water retardation. But addition of sorbitol did not show such a trend. With the addition of 0.1% of sorbitol WVP decreased to 0.009 g.m/m^2.day.kPa, but upon further addition (0.5%) the WVP showed 0.0163 g.m/m^2.day.kPa, which is higher than native chitosan film. Addition of PEG showed a significant increase. At 0.2% the WVP was 0.019 g.m/m^2.day.kPa, whereas with further addition no significant differences were observed. This increase may be due to destabilization of chitosan matrix by long chain PEG molecules, thus widening the interstitial space in the chitosan matrix and allowing for an increased diffusion rate of water molecules through the films.

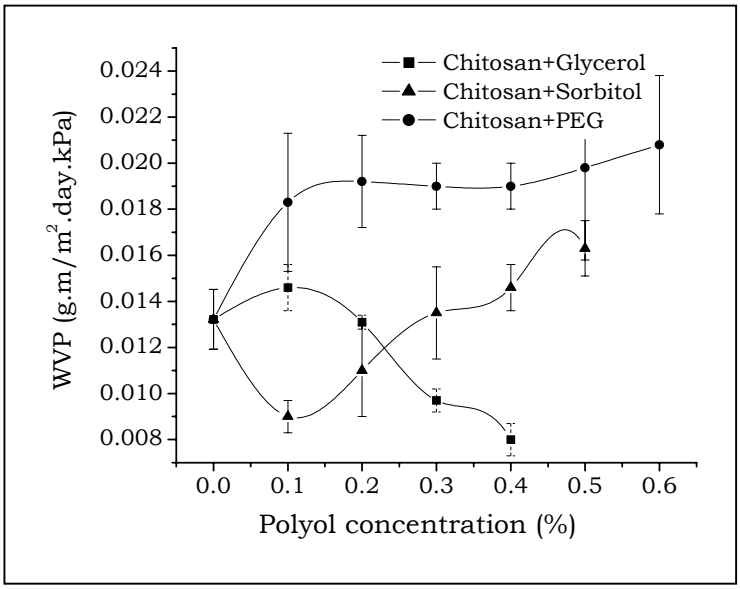


Fig. 3.8. Water vapor permeability of Chitosan-Polyol blend films

Caner *et al.* (1998) reported that addition of PEG to chitosan films increases WVP. Butler *et al.* (1996) reported a mean WVP ranging from 7.6 x 10^{-2} to 1 x 10^{-1} g/m.day.atm. of glycerol blend films, as also reported earlier.

Arvanitoyannis *et al.* (1987) reported an increased WVP with the addition of glycerol, which is contrary to our result, where a decrease in WVP was observed. This may be due to the intrinsic chemical nature of chitosan and also to the way the blend films are prepared. McHugh *et al.* (1994) reported that addition of plasticizer also affects the WVP of whey protein films.

Interaction between packaging materials and food stuff can affect food quality. Gas permeability of food packaging materials is of great importance for food preservation (Arvanitoyannis *et al.*, 1998). The oxygen transmission rate of the plasticized and non-plasticized chitosan films all dried under infrared heating is shown in Fig. 3.9. Addition of glycerol and sorbitol into chitosan films increased the oxygen transmission rate, whereas addition of PEG it was decreased. With the addition of glycerol and sorbitol, the OTR increased, 98.01 and 141.14 x 10^{-6} cc.m/m^2.day.kPa, respectively at

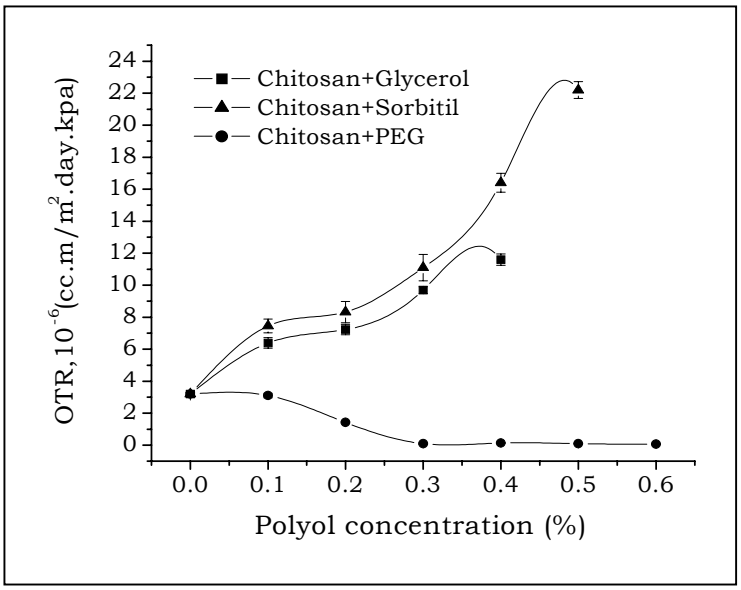


Fig. 3.9. Oxygen transmission rate of Chitosan-Polyols blend films

optimized concentration. In comparison to PEG blend films, sorbitol and glycerol blend films were observed to be better barrier to oxygen (Fig. 3.9). This result is not surprising, since plasticization results in increased mobility

of polymer chains and thus decreased resistance of films to gas transmission. Glycerol would be expected to produce a larger increase in OTR when incorporated into a chitosan film.

The OTR values were decreased drastically with the addition of PEG. Incorporation of PEG may also create a significant increase in crystalline spacing and this may facilitate diffusion through the expanded matrix with chitosan in a manner that restricts the flow of O_2. Several factors may be responsible for differences in OTR of the plasticized films. These include the physical state of the plasticizer, molecular weight, altered film structure due to chemical interaction of plasticizer, and absorption of oxygen molecule. Any increase in the oxygen permeability that might have been anticipated because of glycerol fluidity and ability to expand chitosan matrix may have been off set by good oxygen barrier property of glycerol itself and by the ability of this small molecule to effectively fill in small voids in the polymer matrix.

FTIR studies

Plasticizer addition to chitosan solution and the IR spectra of resulting films showed a major disruption in polymer hydrogen bonding (between O…..H..(H_2O) (Fig.3.10). Glycerol and sorbitol blended with chitosan gives good elongation property. Addition of 0.1% glycerol disturbs H-bonding, which increases as glycerol concentration increases. But higher concentration (0.4%), again due to reorientation of chain, stabilizes the polymeric structure as shown in Fig.3.10, whereas sorbitol (0.4%) has not much affect at the same concentration. But above 0.7% results in extensive disruption. These two polyols do not have much affect on the rest of the basic chitosan structure for ex., amide absorption remains undisturbed. The blending of polyethylene glycol (PEG) in different concentrations with chitosan solution showed subtle differences in the IR spectra, 0.2% addition resulted in disturbance in hydrogen bonding of chitosan chain in which PEG

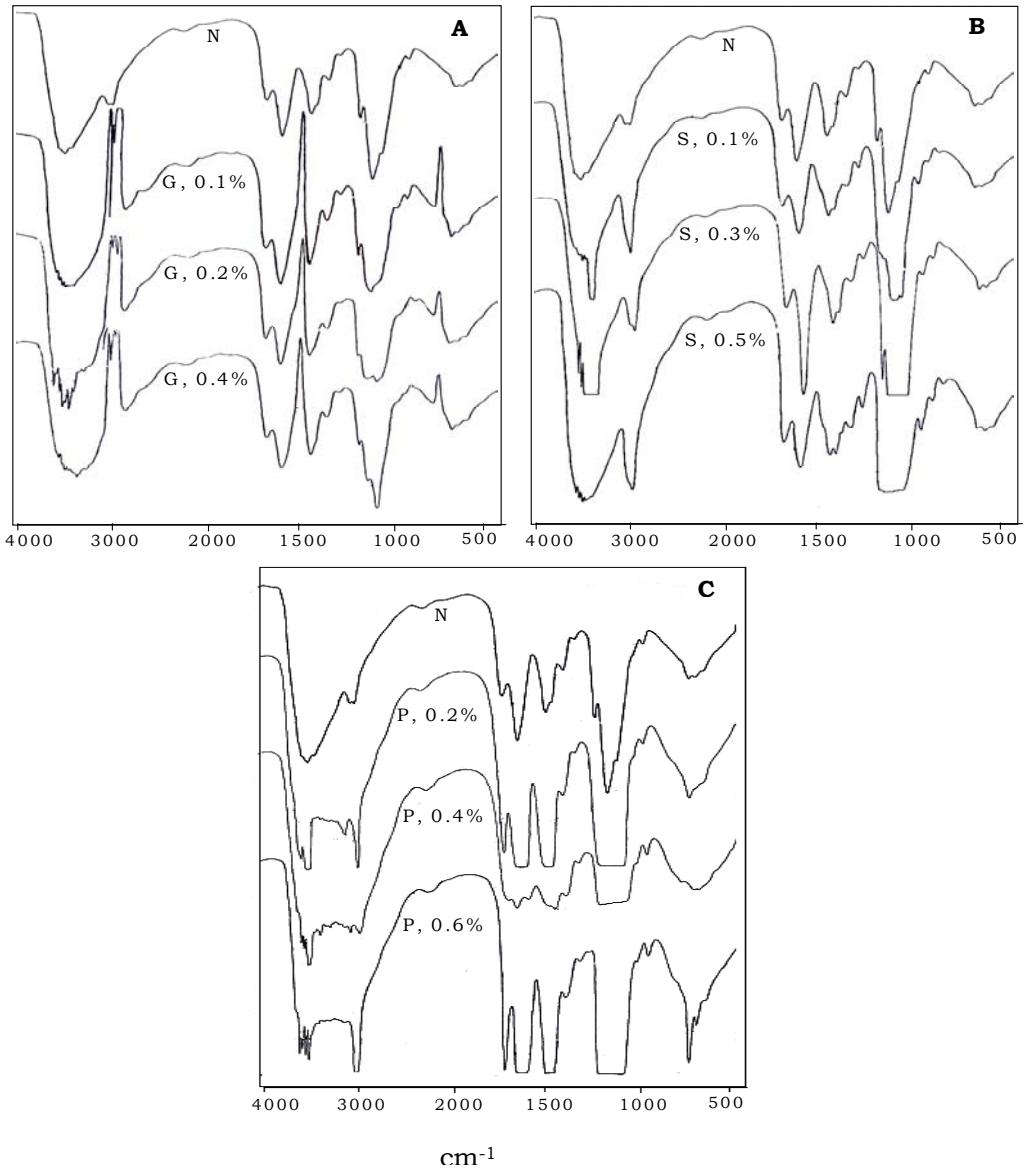


Fig. 3.10. FTIR spectra of Chitosan-Polyol blend films-A with glycerol; B with sorbitol; C with PEG

being a polymeric plasticizer introduces itself into the crevices of the chain, interfering with intra- and inter-H- bonding of chitosan, but as the concentration of PEG increases there will be rearrangement of chitosan and PEG chains. Absorption around 2875 cm $^{-1}$ due to $-CH_2$ and 1560 cm^{-1} due to glycerol mobility increased as the concentration increases. 1560 cm^{-1} shifts to 1565 cm^{-1} for 0.6% of PEG addition. The orientation of the chains as the PEG concentration changes is also evident by the appearance of absorption peaks at 948 cm^{-1}.

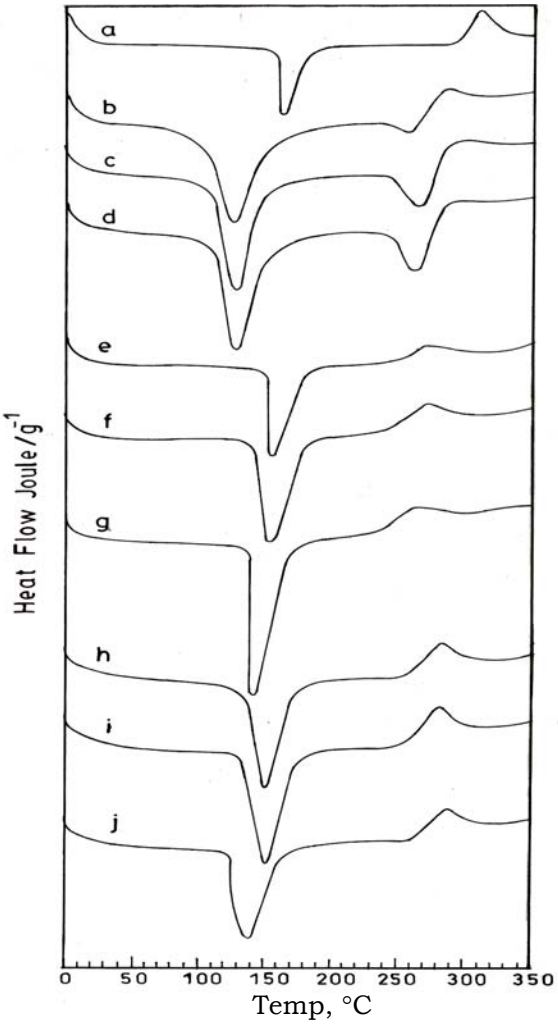
Thermal properties

The normalized DSC thermograms of chitosan film and the various blend films are shown in Fig. 3.11. and the enthalpy change (ΔH) and decomposition temperature values are shown in Table 3.3. The thermogram showed a difference in the ΔH values for various polyols. For glycerol a distinct endotherm peak appeared around 260°C, which is due to glycerol decomposition. ΔH values increased as glycerol concentration was increased in the blend films, and it attained saturation in the range of ΔH –170 J/g. But the endotherm attributable to other two polyols viz., sorbitol and PEG was not conspicuous, even at higher concentration levels. Instead they showed a typical chitosan thermogram having an endotherm around 150°C (due to water holding capacity of chitosan) and an exotherm in the range 270-290°C as a function of decomposition.

Table 3.3. Melting and heat of fusion of Chitosan-Polyol blend films

Conc., %		Endotherm		Endotherm		Exotherm	
		ΔH J/g	T_p ,°C	ΔH J/g	T_p , °C	ΔH J/g	T_p, °C
Chitosan		-286.11	140.43	-	-	166.54	297.71
Glycerol	0.1	-462.94	123.46	-90.40	258.56	120.77	285.77
	0.2	-448.08	126.89	-172.58	262.43	167.92	288.33
	0.4	-468.69	128.19	-172.54	263.72	191.19	287.00
Sorbitol	0.1	-451.04	157.84			68.9	275.38
	0.3	-366.34	157.94			92.09	276.32
	0.5	-337.57	144.74			56.27	268.66
PEG	0.2	-385.17	152.05			93.31	292.81
	0.4	-388.48	153.45			108.38	291.87
	0.6	-361.08	142.09			83.15	291.60

Water capacity of the blend films showed different characteristics, that of glycerol showed early water evaporation around 125° C with high ΔH values (around 460 J/g), compared to other two plasticizers (ΔH values around 380 J/g). The exothermic temperature at around 285° C showed increase in ΔH values for film blended with glycerol, whereas for PEG and sorbitol blend films ΔH values showed initial increase and sudden decrease as the concentration of respective polyols was increased. PEG blended chitosan films showed a little higher decomposition temperature (around 290°C) due to its higher molecular weight. Increased ΔH values for glycerol blend films supports our earlier discussion on their WVP properties.



a. Chitosan
b. Chitosan + 0.1 %Glycerol
c. Chitosan + 0.2 % Glycerol
d. Chitosan + 0.4 % Glycerol
e. Chitosan + 0.1 % Sorbitol
f. Chitosan + 0.3 % Sorbitol
g. Chitosan + 0.5 % Sorbitol
h. Chitosan + 0.2 % PEG
i. Chitosan + 0.4 % PEG
j. Chitosan + 0.6 % PEG

Fig. 3.11. DSC of Chitosan-Polyol blend films

X-ray diffraction

X-ray diffraction pattern of native chitosan showed (Fig.3.12) usual hydrated 10° reflection peak around 11.92° and a blunt peak around 15° , which is 120 reflection, due to anhydrous crystal lattice (Ogawa *et al,* 1992). The latter is characteristic of annealed polymorph. This was possible due to the preparation process involving higher temperature (IR drying) of the cast films.

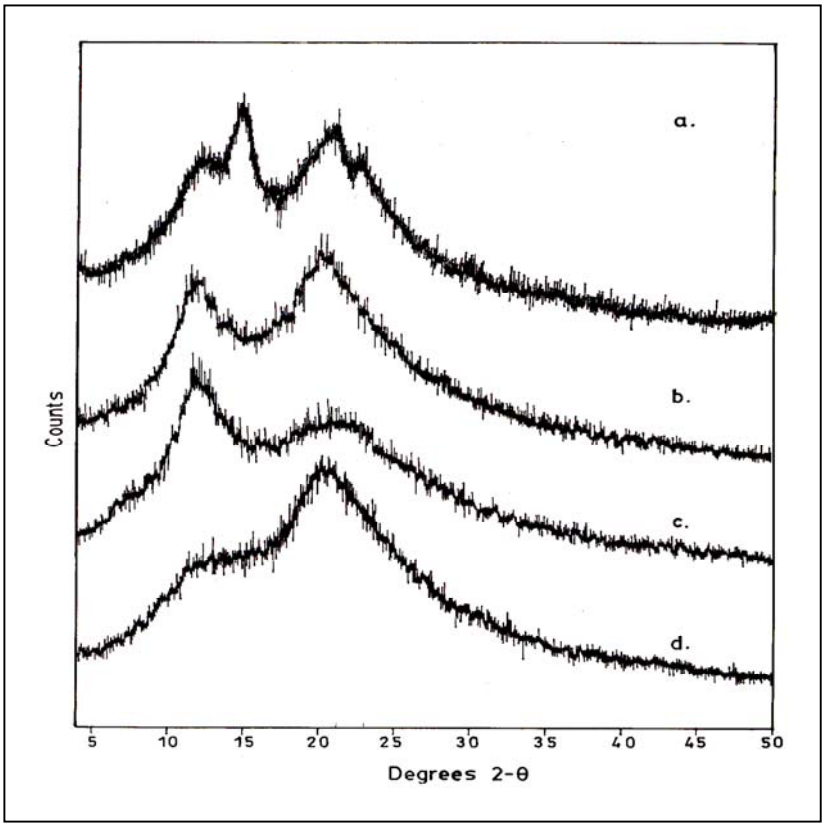


Fig. 3.12. X-ray diffraction patterns of Chitosan-Polyols blend films. a- native chitosan film; b- with glycerol; c-with sorbitol; d with PEG

Different plasticizers have been added to note changes in diffraction pattern of the modified chitosan films. Donhowe and Fennema (1993b) had shown that the intensity of diffraction peak (d_{101}) did not change significantly, instead there was an increase in d spacing value (peak shift) in the case of plasticized methylcellulose films, depending on the molecular

weight of the plasticizer. Contrary to this, our data revealed plasticizer dependent differences in intensities of respective peaks. However, there was no significant change in peak position when chitosan was blended with glycerol, sorbitol and PEG. Addition of glycerol, created hydrated crystals thereby giving rise to "tendon" form, where 2θ at 11.92° got shifted to 11.6° and the relative intensity increased from 70 to 90%. Sorbitol addition showed another 10% increase in its relative intensity at 10^0 reflection. However, there was no significant shift in its d spacing value (7.363 Å). But the peak around 20° showed decreased intensity, may be due to reduction of spacing in the crystalline region by sorbitol-chitosan molecular interaction and respective hydrogen bonding. PEG, increased the d spacing value (7.762 Å) much more compared to glycerol, but its intensity was 65% reduced due to its higher molecular weight, which influences in increased chain mobility and in turn increases loss of water holding capacity in hydrated crystals.

Even though IR drying is rapid and would likely enhance the crystallinity of the chitosan film, the anhydrous crystals formation affects the chemical and biological nature of the films. Whereas the addition of polyols completely overcomes this defect and restores chitosan film in a hydrated form. Glycerol proved to be the best compared to other polyols. Miscibility of polyols in viscous chitosan solution is another important factor while preparing the blend films.

B. Fatty acids

Fatty acids are hydrophobic in nature. The density of fatty acid blend films decreased (Fig. 3.13), it ranged from 1.4024 to 1.2692 g/ml in palmitic acid, whereas in stearic acid it was 1.4024 to 1.2585 g/ml, not much difference was observed between the two acids. The addition of fatty acid increased the colour of the film from light yellow to whitish yellow

Table 3.4. Chitosan films blended with fatty acids - Colour, opacity and WRV

Palmitic acid	Conc., (%)	L	a	b	Opacity	WRV
	0.0	95.84 ± 1.48	-1.20 ± 0.23	8.26 ± 1.23	8.11 ± 0.23	6.04 ± 0.3
	0.1	95.89 ± 1.07	-1.52 ± 0.16	3.48 ± 0.18	12.48 ± 1.15	8.20 ± 1.20
	0.2	95.76 ± 0.35	-1.79 ± 0.43	4.76 ± 1.41	12.90 ± 0.75	6.78 ± 1.67
	0.3	93.74 ± 1.56	-1.93 ± 0.22	3.59 ± 0.24	12.98 ± 2.34	5.90 ± 0.98
	0.4	95.49 ± 012	-1.78 ± 0.34	4.89 ± 0.37	20.01 ± 1.76	5.34 ± 1.08
	0.5	95.49 ± 0.28	-2.08 ± 0.14	5.89 ± 0.46	27.43 ± 1.56	4.19 ± 1.60

Stearic acid	Conc., (%)	L	a	b	Opacity	WRV
	0.0	95.84 ± 1.48	-1.20 ± 0.23	8.26 ± 1.23	8.11 ± 0.23	6.04 ± 0.3
	0.1	95.89 ± 1.07	-1.52 ± 0.16	3.48 ± 0.18	8.39 ± 0.81	6.24 ± 0.75
	0.2	95.76 ± 0.35	-1.79 ± 0.43	4.76 ± 1.41	8.48 ± 0.23	5.89 ± 1.03
	0.3	93.74 ± 1.56	-1.93 ± 0.22	3.59 ± 0.24	9.06 ± 0.19	5.20 ± 0.43
	0.4	95.49 ± 012	-1.78 ± 0.34	4.89 ± 0.37	8.15 ± 0.46	5.01 ± 0.76
	0.5	95.49 ± 0.28	-2.08 ± 0.14	5.89 ± 0.46	8.28 ± 0.66	4.98 ± 0.32

colour, due the nature of the fatty acid as mentioned in Table 3.4. The opacity of the palmitic acid blend film increased from 12.20 % to 24.23 % at 0.5%, whereas in stearic acid blend films the values were ranging from 10.43 to 23.65%. Again not much difference was observed between the two fatty acid blend films (Table 3.4).

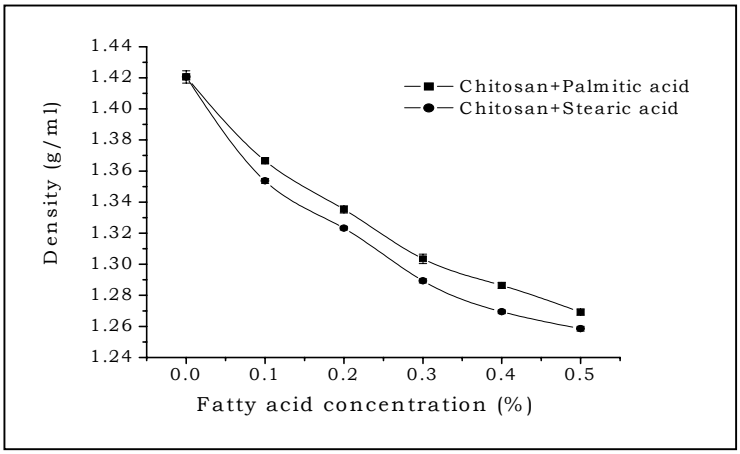


Fig.3.13. Density of Chitosan -Fatty acid blend films

Mechanical properties

The TS, %ε and ME of fatty acid blend films are shown in Fig. 3.14-3.16. The TS was 9.16 MPa for 0.1% of palmitic acid, with further addition (0.5%) it increased to 20.48 MPa. With stearic acid blend films not much changes in TS were observed. Its TS ranged from 16.22-13.2 MPa, significantly less compared to native chitosan films (39.1MPa).

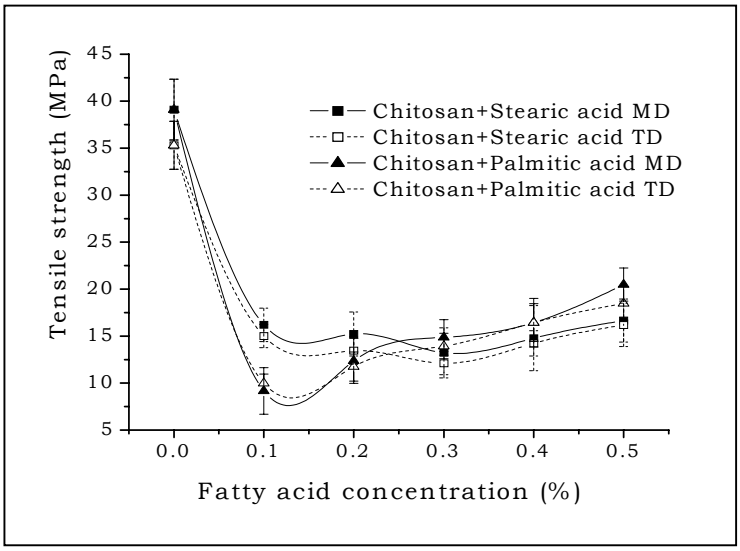


Fig.3.14. Tensile strength of Chitosan -Fatty acid blend films.

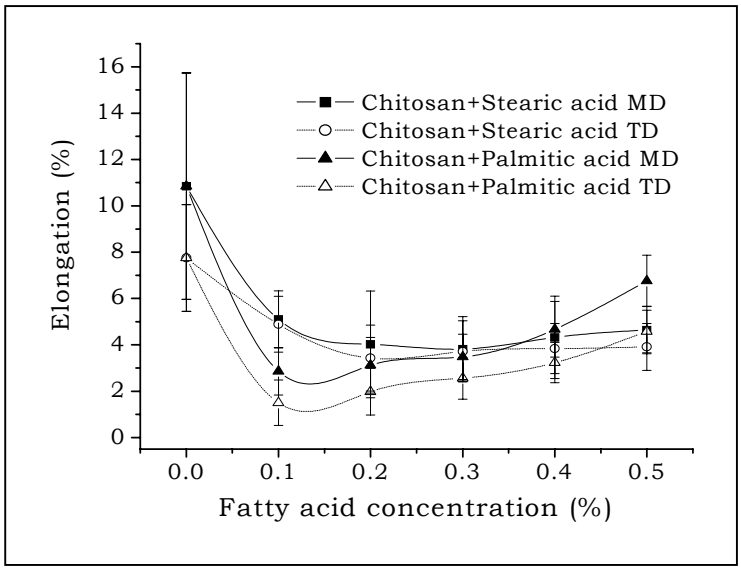


Fig. 3.15. % Elongation of Chitosan -Fatty acid blend films

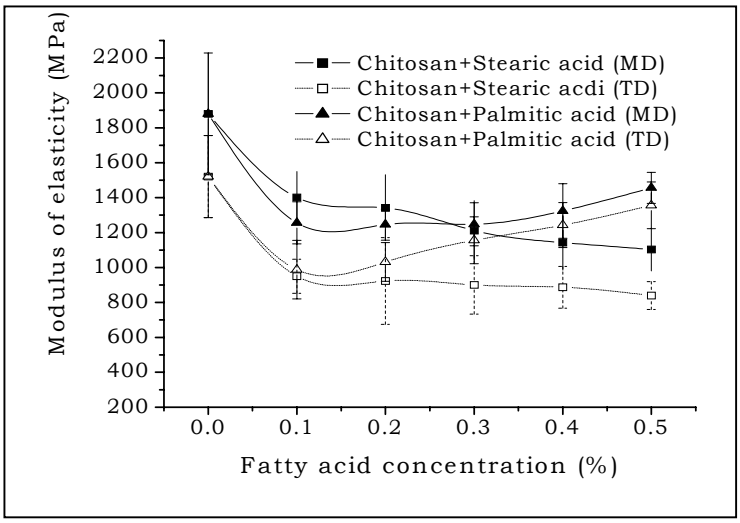


Fig. 3.16. Modulus of elasticity of Chitosan-Fatty acid blend films

Shellhammer and Krochta (1997) reported decrease in TS of protein-lipid films. Only small differences in %ε at break (Fig.3.15) were seen, and all the values were lower than of native films. In stearic acid blend films the %ε was reduced by 50%. But no significant differences were observed between three concentrations, the values ranged from 6-3%. With the addition of fatty acid the TS and %ε of the film decreased. ME showed a decreasing trend with the addition of fatty acid. In stearic acid the ME values ranged from 1147-779 N/mm^2, no difference was observed between 0.3 and 0.5% concentration.

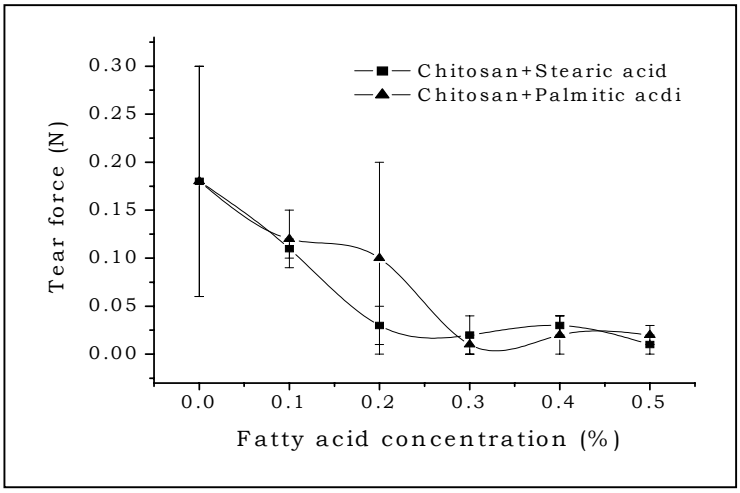


Fig. 3.17. Tearing strength of Chitosan -Fatty acid blend films

In tear propagation strength (Fig 3.17), no significant differences were found between two fatty acids. The higher variation observed may be due to the undissolved fatty acids at higher concentration and test specimens taken for trails. Burst strength and impact strength of fatty acid blend films are shown in Figs.3.18 and 3.19. Burst strength showed a decrease due to brittleness of the film. Similar result was also observed in impact strength.

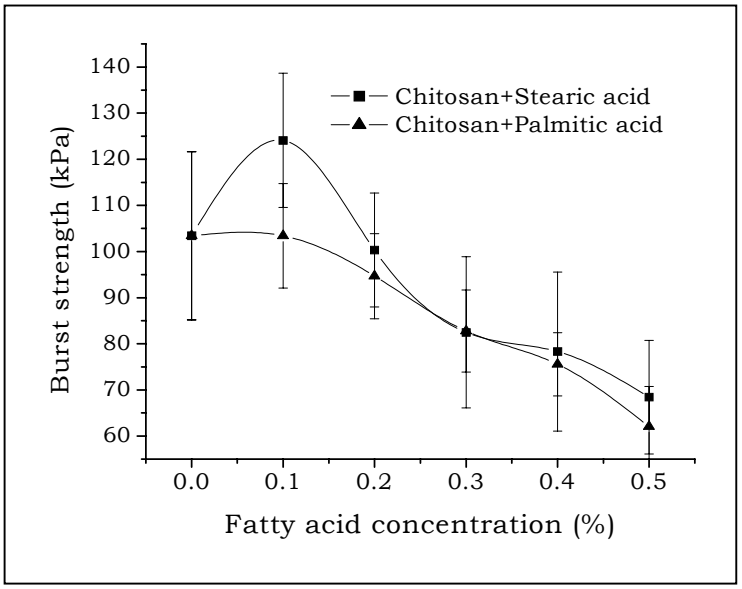


Fig. 3.18. Burst strength of Chitosan -Fatty acids blend films

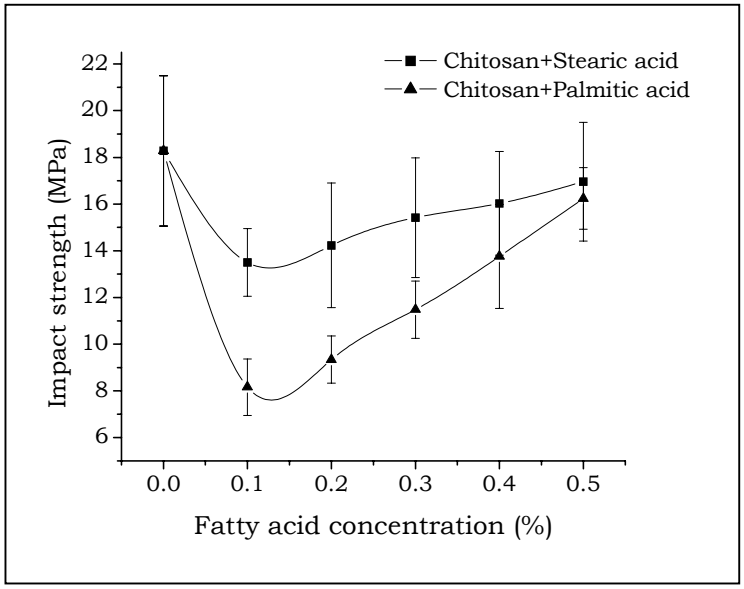


Fig. 3.19. Impact strength of Chitosan -Fatty acid blend films

Barrier properties

The WVP of the native chitosan film was 0.01322 g.m/m^2.day.kPa, no considerable differences were observed with fatty acid (stearic/palmitic acids) blend chitosan films (Fig. 3.20). The WVP varied from 0.0125 to 0.0168 g.m/m^2.day.kPa.This result is supported by the work of Wong *et al.* (1992), where WVP and OTR properties of chitosan-lipid blend films were comparable. Plamitic acid blend film showed a high WVP compared to native films. This may be attributed to the cationic nature of chitosan as well as the hydrophobicity of the blend films, wherein the water molecules may interact with matrix and increase the permeation rate (Pascat, 1986). Kamper and Fennema (1984) studied the WVTR of edible film blended with fatty acid, and reported that higher concentration of fatty acid will not affect the WVTR of blend films, but coating of fatty acid on edible film affects the WVTR.

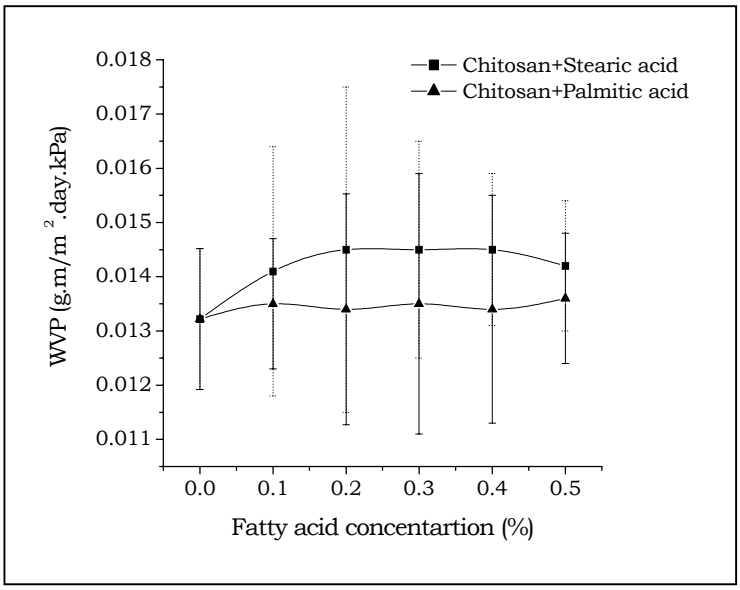


Fig. 3.20. Water vapor permeability of Chitosan -Fatty acid blend films

Oxygen Transmission Rate (OTR)

By nature, biopolymers offer potential barrier to gases. The OTR results (Fig. 3.21) showed that the addition of fatty acids decreases gas transmission rate, and no significant differences were observed between the

two fatty acids. At 0.1% of stearic/palmitic acid there was drastic reduction in OTR from 3.2 to 0.0639 cc.m/m^2.day.kPa, but at optimized levels it was 0.0234 cc.m/m^2.day.kPa, in accordance with the earlier report (Wong *et al.,* 1992). The variation in permeability characteristics is ascribable to subtle microstructure of the films, viz increase in density, pore formation, channeling and packing pattern of the lipids in the blend films. Transmission of gases through packed polymer is lowest when compared with that in water or air, with differences of 3 orders of magnitude (Mannapperuma and Singh, 1990).

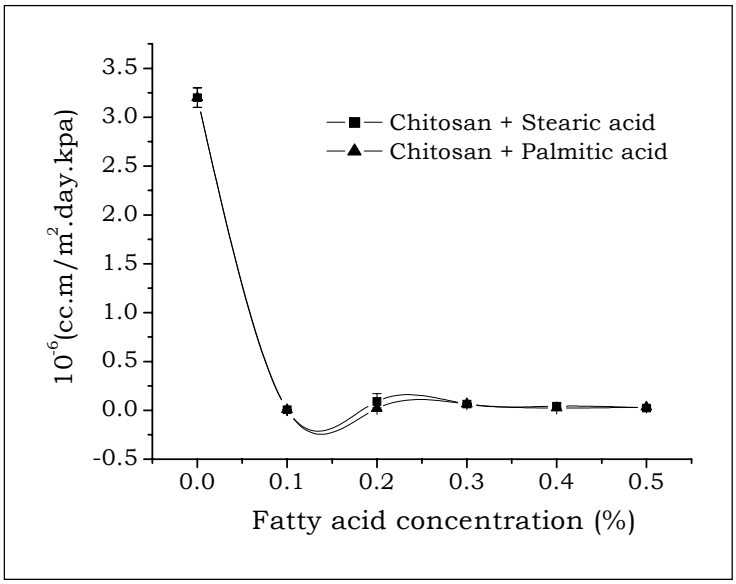
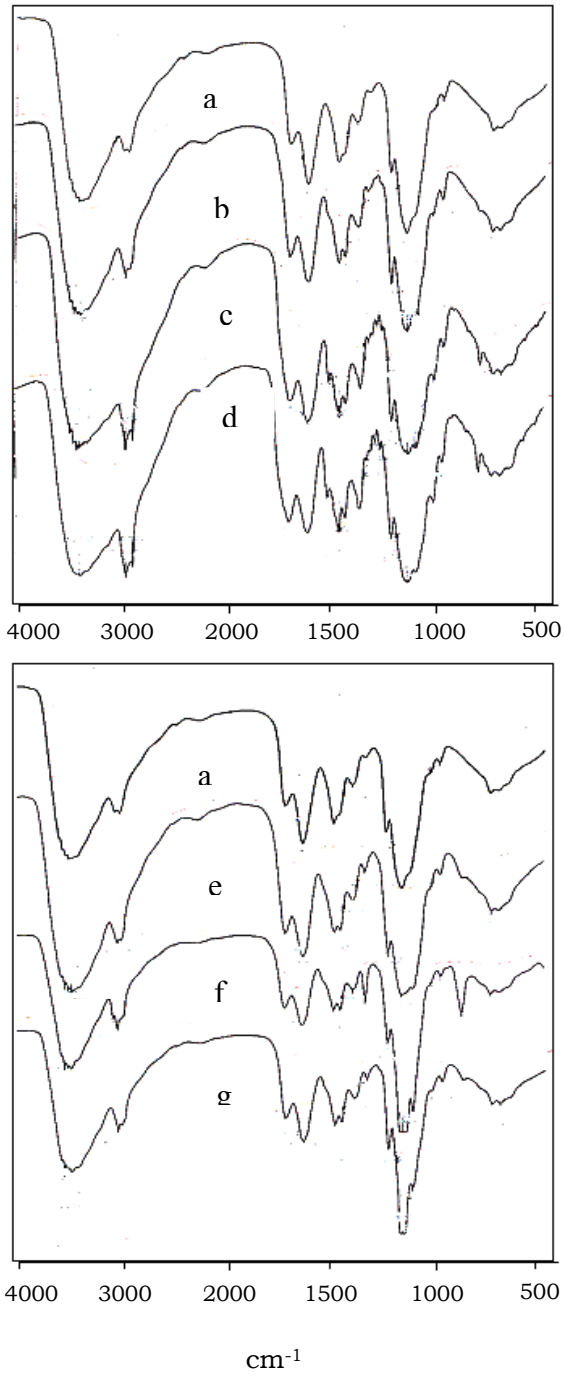


Fig. 3.21. Oxygen transmission rate of Chitosan -Fatty acid blend films

FTIR studies

Addition of palmitic and stearic acids results in more sharper overall absorption peaks (Fig.3.22) indicating the increase in hydrophobicity due to (fatty acids) which films results in increased thermal stability. The hydrogen bonds between hydroxyl groups and water molecules remain intact. The methyl and methylene stretching around 2918 and 2850 cm^{-1} clearly indicate palmitic/stearic blending, which resulted in increased amide II stretching.

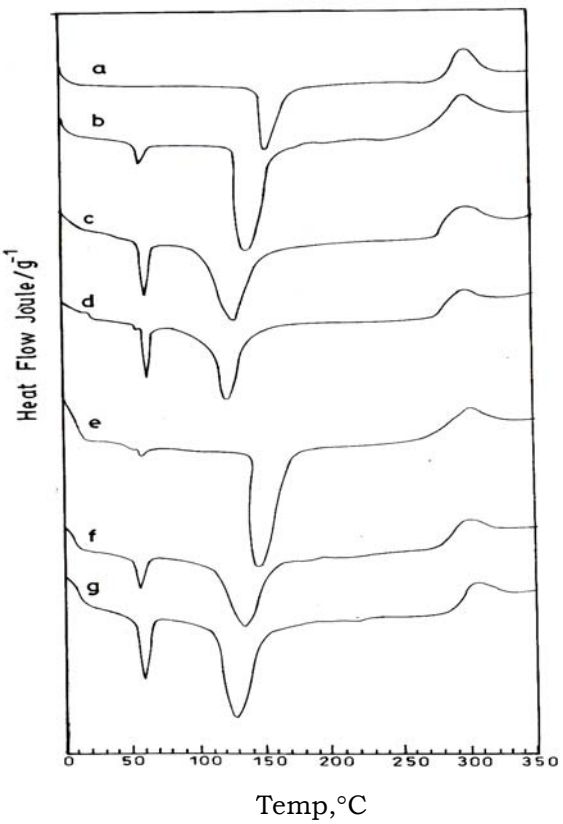


a. Chitosan
b. Chitosan + 0.1 % Stearic acid
c. Chitosan + 0.3 % Stearic acid
d. Chitosan + 0.5 % Stearic acid
e. Chitosan + 0.1 % Palmitic acid
f. Chitosan + 0.3 % Palmitic acid
g. Chitosan + 0.5 % Palmitic acid

Fig. 3.22. FTIR spectra of Chitosan-Fatty acid blend films

Thermal properties

Palmitic and stearic acid blend chitosan films show melting peaks around 63° C and 56°C, respectively (Fig.3.23). As their concentration increased there was a steep increase in ΔH values (Table 3.5). Stearic acid blend film obviously showed a maximum of –72.63 J/g. at 0.5% concentration, its long carbon chain enhanced the hydrophobicity, resulting in decrease in the endotherm peak around 130^{0}C. However, noticeable water content was found in these films (in the range of 270 to 385 J/g). Due to the complete decomposition of fatty acids at higher temperature, the exotherm of chitosan decreased to 300° C, indicating a sort of superficial blending unlike in polyol blending where it resulted in a shift towards a lower temperature. Exothermic ΔH values decreased as fatty acid concentration increased.



a. Chitosan
b. Chitosan + 0.1% Stearic acid
c. Chitosan + 0.3 % Stearic acid
d. Chitosan + 0.5 % Stearic acid
e. Chitosan + 0.1 % Palmitic acid
f. Chitosan + 0.3 % Palmitic acid
g. Chitosan + 0.5 % Palmitic acid

Fig. 3.23. DSC thermogram of Chitosan -Fatty acid blend films

Table 3.5. Melting and heat of fusion of Chitosan-Fatty acid blend films

Concentration (%)		Endotherm		Endotherm		Exotherm	
		ΔH J/g	T_p, ^oC	ΔH J/g	T_p, ^oC	ΔH J/g	T_p, ^oC
Chitosan				-286.11	140.43	166.54	297.71
Palmitic acid	0.1	-11.92	63.32	-385.24	139.39	119.48	299.33
	0.3	-34.18	64.19	-335.98	124.81	94.27	295.57
	0.5	-35.46	63.72	-269.95	132.02	56.58	297.40
Stearic acid	0.1	-13.68	56.97	-378.81	144.64	68.9	275.38
	0.3	-29.20	57.18	-304.92	134.64	92.09	276.32
	0.5	-72.63	57.97	277.10	126.30	56.27	268.66

X-Ray diffractometry

When the fatty acids were added in different ratios the conversion of anhydrous form may be avoided as evident from the spectra of blend films. Due to hydrophobic interaction of fatty acids with chitosan the blend films tends to hold water molecules even after drying, leading to conversion of broad peak of 10° (i.e. around 11.92 ° in chitosan) to sharp crystalline peak (Fig.3.24). The additional peak due to fatty acid incorporation can be assigned at 6.74 ° for stearic and 7.35 ° for palmitic acid and showed addition of palmitic acid give more crystalline nature to films when compared to stearic acid.

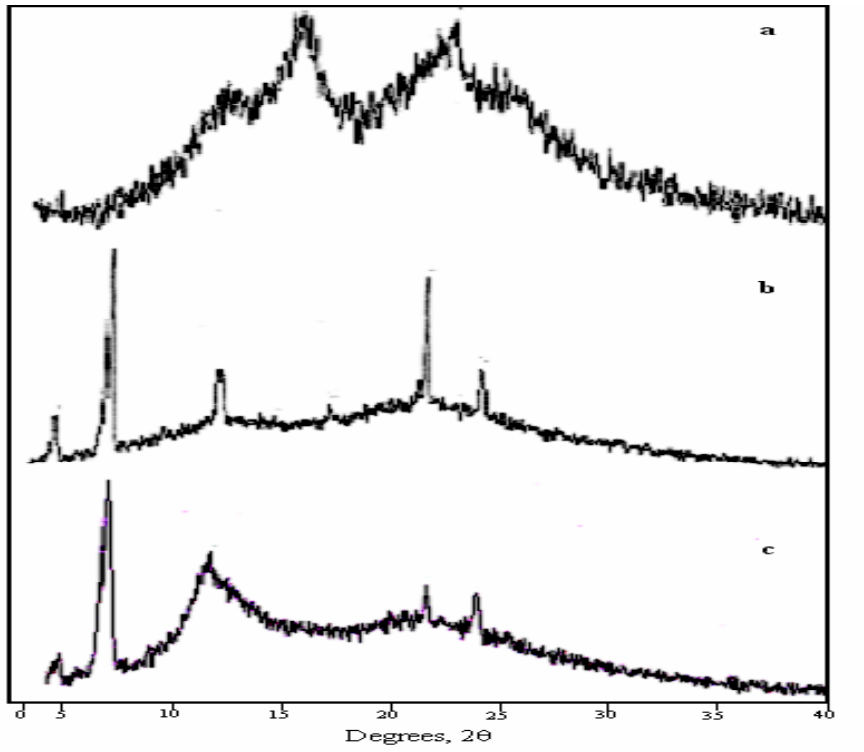


Fig. 3.24. X-ray diffraction pattern of Chitosan -Fatty acid blend films. a Chitosan films ; b. with stearic acid; c. with palmitic acid

C. Synthetic water-soluble polymer

PVA, the synthetic water-soluble polymer with high crystallinity and having a planar zigzag conformation is easily miscible with chitosan solution. The density of chitosan–PVA blend film decreased from 1.4021 to 1.1680 g/ml (Fig.3.25). Being more hydrophilic in nature, PVA tends to increase water uptake of the blend films. The water retention value (WRV) of blend films is shown in Table 3.6. WRV increased with the addition of PVA to native chitosan film. WRV of native PVA could not be determined since it is soluble in water.

Table 3.6. Optical properties and water retention value of Chitosan-PVA blend films

Chitosan-PVA	L	a	b	Opacity	WRV
100-0	92.48 ± 1.48	1.20 ± 0.23	8.26 ± 1.23	8.11 ± 0.23	6.08 ± 0.3
80-20	93.26 ± 1.20	-0.65 ± 0.12	1.72 ± 0.24	7.93 ± 0.13	6.35 ± 0.53
60-40	94.48 ± 1.26	-0.58 ± 0.25	1.19 ± 0.25	7.79 ± 0.16	6.84 ± 0.54
40-60	95.31 ± 1.27	-0.59 ± 0.23	0.79 ± 0.45	7.56 ± 0.32	7.06 ± 0.27
20-80	93.36 ± 1.32	-0.56 ± 0.17	0.78 ± 0.32	7.34 ± 0.21	7.20 ± 0.27
0-100	97.51 ± 1.72	-0.32 ± 0.12	-0.07 ± 0.02	7.07 ± 0.24	ND

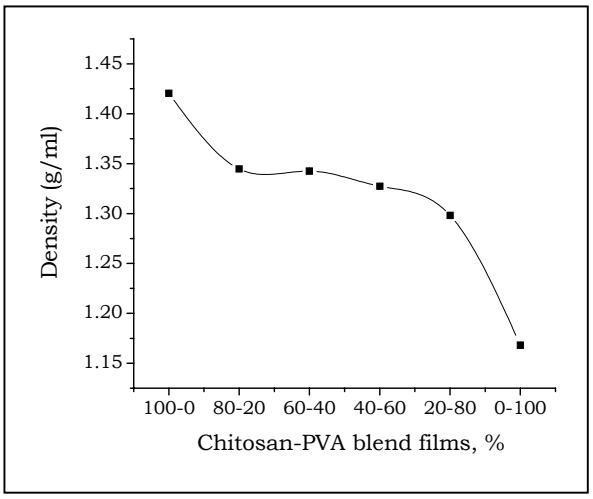


Fig. 3.25. Density of Chitosan-PVA blend films

The swelling ratio is linearly related to the weight fraction of PVA, as also observed by Nakasuka and Andrady (1992). The Hunter L, a, b values of the blend films Table 3.6 showed increase in lightness with the addition of PVA. Colour of the packaging is an important factor in terms of general appearance and consumer acceptance. The main difference observed was that films with higher concentration of PVA had lighter colour as indicated in L values. The L values ranged from 92.48 for chitosan and 97.51 for native PVA. The chitosan films are whitish yellow coloured as indicated by 'b', (yellowness) values, which showed an increase from -0.31 for PVA to 8.26 for chitosan films. Similar changes were also observed in Hunter 'a' values.

Chitosan film showed a higher opacity value (8.11%) and as chitosan concentration decreased the value also decreased and lowest value was observed in PVA film (7.03%).

Mechanical properties

Tensile strength (TS) of the PVA blend films showed a dual trend (Fig.3.26). Native chitosan and PVA showed strength of 39.2 and 26.47 MPa, respectively. The TS of blend films showed a decreasing trend with increase in PVA concentration; at 60-40 ratio it showed maximum TS (41.14 MPa). The slight increase in TS may be due to reduction in crystallinity of the blend films (Blair *et al*. 1987). Miya *et al*. (1983) studying the properties of chitosan -PVA blend films found that the presence of PVA molecule in a chitosan system tended to disrupt crystallinity of chitosan, which increases the amorphous content. Nakatsuka and Andrady (1992) reported that hydrogen-bonding interaction between chitosan–PVA does not lead to tighter network structure. The result is supported by the work of Park *et al*. (2001) where 80-20 blend is having a better property than 60-40 blend. Since there is a sudden decrease in the modulus of elasticity, no significant changes were observed in TS. The lower strength of PVA may also be due to its low degree of polymerization. Hasegawa *et al*. (1992) while studying the cellulose–chitosan blend films, observed that at 40-60 blend ratio the film showed maximum TS compared to native and other blend films. Blending of polyvinylpyrrolidone (PVP) with chitosan resulted in decrease in TS, due to the presence of amorphous and hydrophilic PVP (Qurashi *et al.*, 1992).

The results of % elongation at break showed that chitosan films had lower value (8%) compared to PVA films (120%). % elongation of blend films increased with addition of PVA (Fig.3.27), probably attributed to hydrogen bonding between OH group of PVA and amide group of chitosan. The stiffness of the chitosan-PVA blend film decreases with the addition PVA as indicated by ME (Fig.3.28).

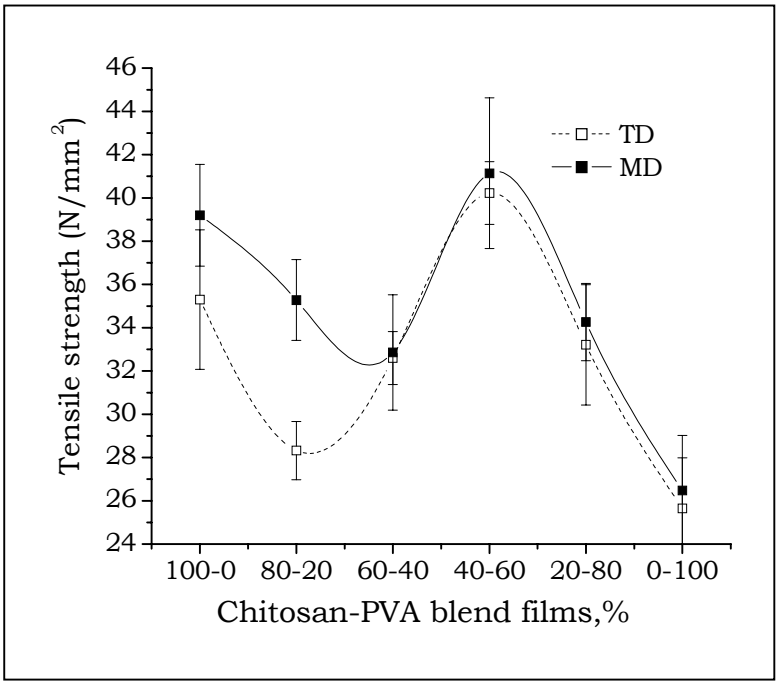


Fig.3.26. Tensile strength of Chitosan-PVA blend films

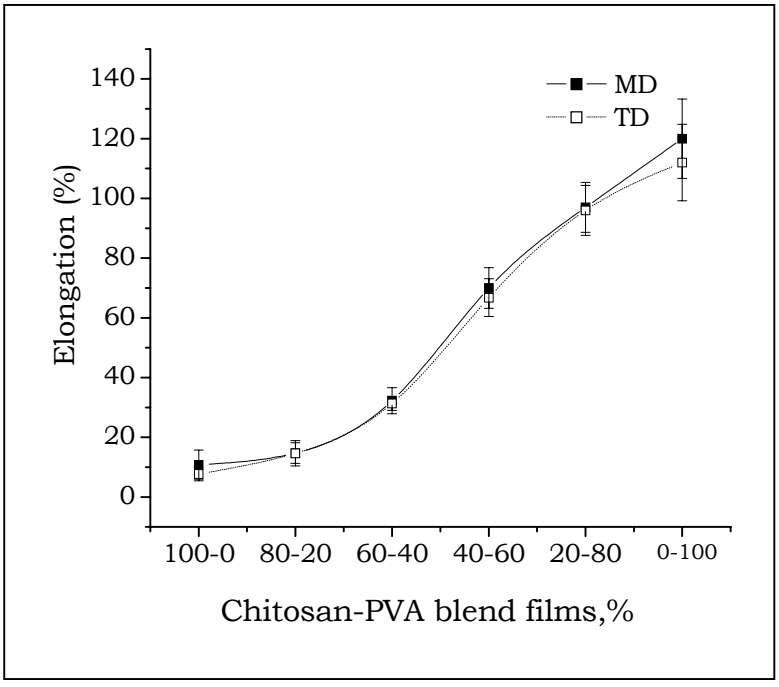


Fig.3.27. % Elongation of Chitosan-PVA blend films

With the addition of PVA the tear propagation increased (Fig.3.29). Native PVA had a value of 0.35 N. Even though PVA is a synthetic polymer the tear strength value is very negligible compared to synthetic plastic, may be due to its hydrophilic nature

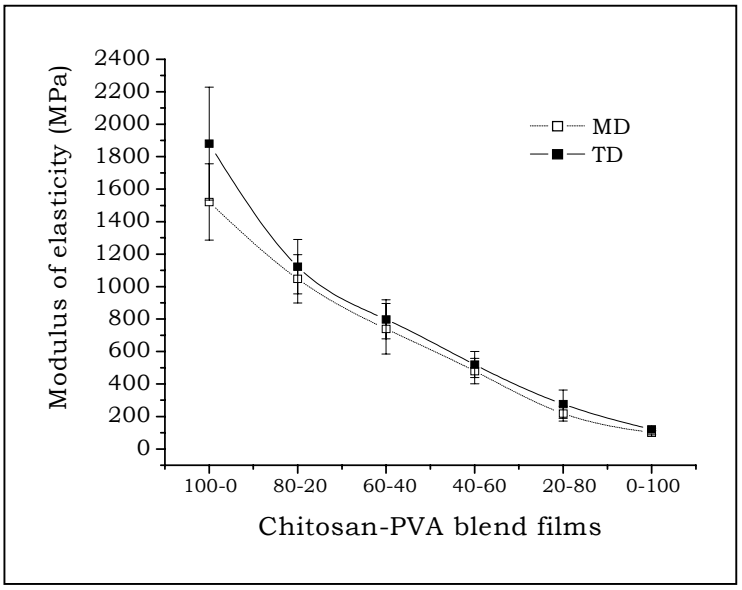


Fig.3.28. Modulus of elasticity of Chitosan-PVA blend films

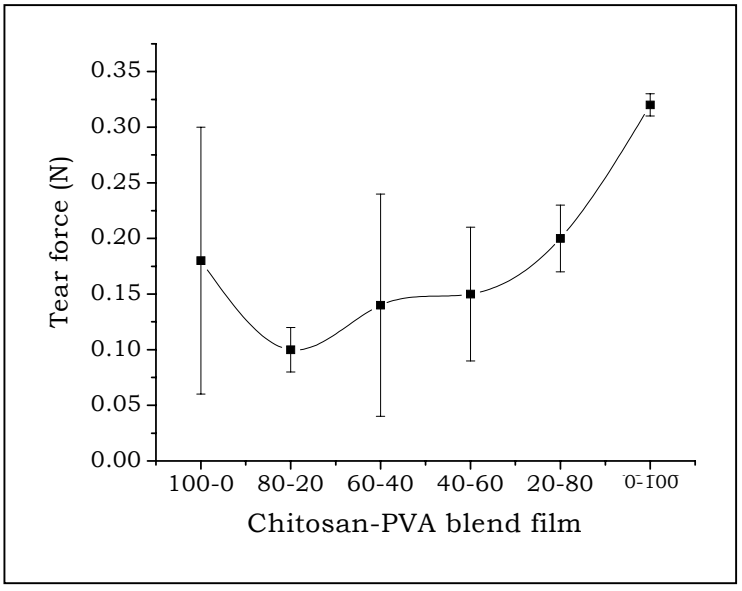


Fig.3.29. Tearing strength of Chitosan-PVA blend films

The Burst and impact strength of the blend films increased with the addition of the PVA (Figs.3.30 and 3.31). Burst strength values ranged from 1.86 to 5 kPa. At 60-40 ratio the blend film showed a value 4.9 kPa, but with further increase of PVA the value remained same. The impact strength values were in the range of 18.28 to 92.52 kPa. With the addition of 40% PVA it increased by over 3 folds (60.08 kPa), but no further difference was observed with further addition of PVA. The increase in the impact strength may be attributed to the chain flexibility of blend films. In general the hydrophilic films were shown to have low impact and burst strength. Chitosan-PVA blend films showed more impact strength compared to polyol blend films, as previously mentioned.

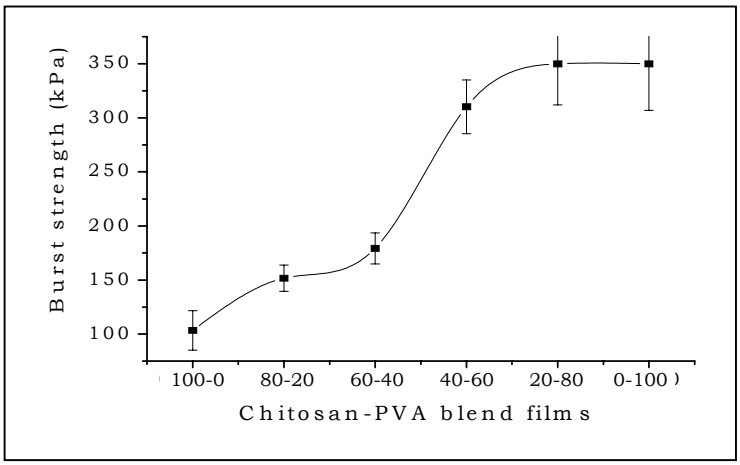


Fig.3.30. Burst strength of Chitosan-PVA blend films

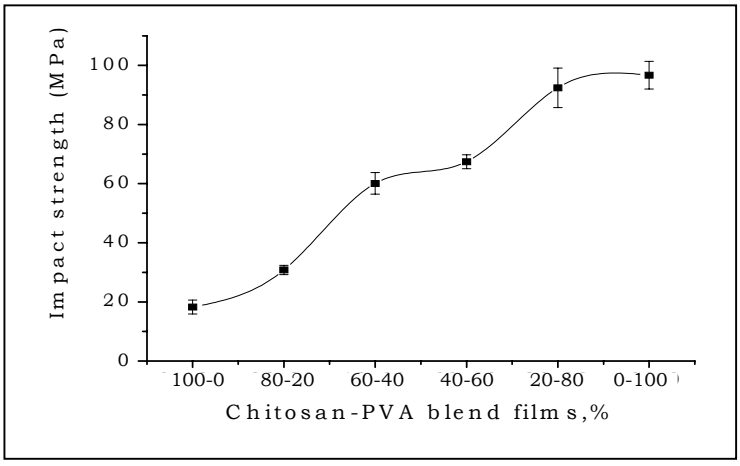


Fig.3.31. Impact strength of Chitosan-PVA blend films

Barrier properties

Barrier to moisture, oxygen, carbon dioxide, aroma and flavor compounds in a packaging system can increase food product shelflife and also improve the food quality (Park *et al.*, 2001). Hydrophilic films have more water vapor permeability (WVP) values. The chitosan film has WVP of 0.01322 g.m/m^2.day.kPa, and with the addition of PVA it got decreased (Fig.3.32). With the addition of small amounts of PVA the rate of decrease in WVP was more. It was observed that a 60-40 blend film showed a gradual decreasing trend in WVP values, probably due to reduced diffusion co-efficient, decrease in crystallinity, and introduction of junction point (Arvanitoyannis *et al.*, 1987).

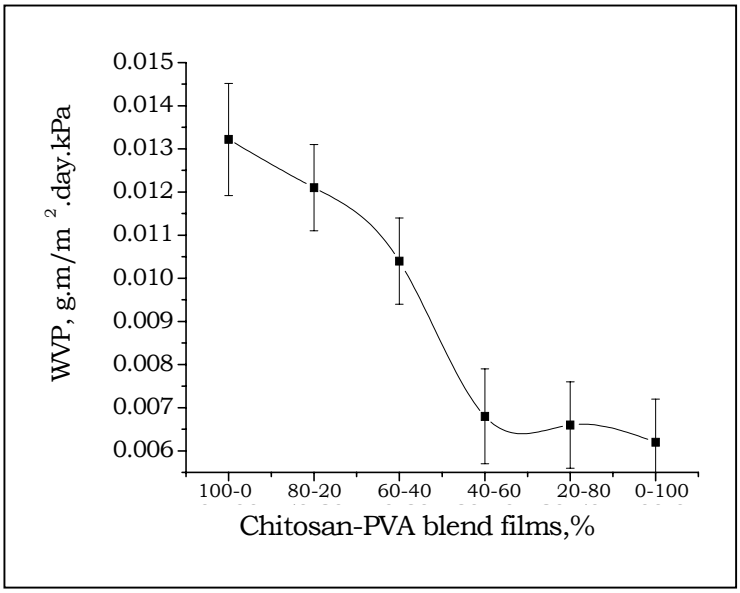


Fig.3.32. Water vapor permeability of Chitosan-PVA blend films

Oxygen barrier property of native chitosan film was much less than synthetic film. Chitosan film had a value of 3.2 x 10^{-6} (cc.m/m^2.day.kPa). Chitosan-PVA blend film showed decrease in OTR (Fig.3.33). With the addition of 20% of PVA drastic decrease in OTR was observed (4.8 x 10^{-7} cc.m/m^2.day.kPa). With further additions the OTR was completely

decreased. Earlier work of Arvanitoyannis *et al.* (1987) on CO_2 sorption and permeability of PVA-chitosan blend films, reported that permeability increases with addition of chitosan. The low OTR in the blend film may be due to the solute presumed to diffuse through the micro-channel or pores within the membrane structure. The water molecule thus fitted in the micro-channel may obstruct the movement of the gas molecule.

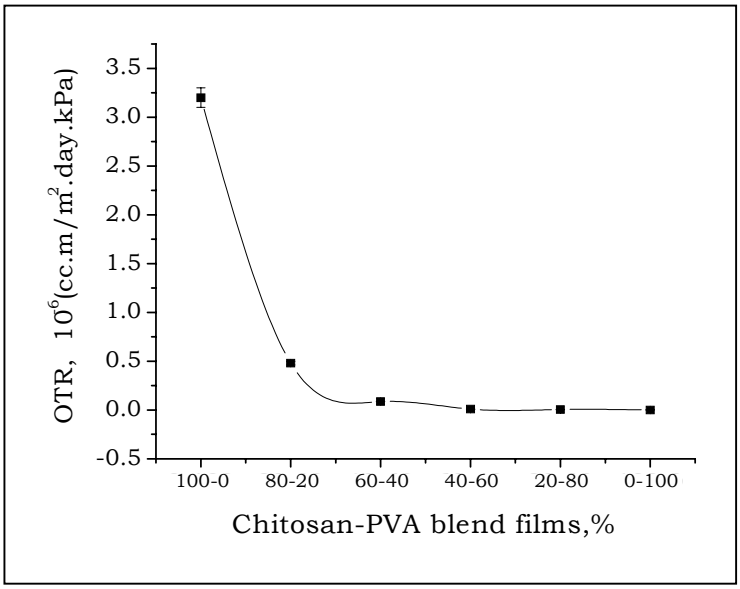


Fig.3.33. Oxygen transmission rate of Chitosan-PVA blend films

FTIR studies

The FTIR spectra of chitosan, PVA and their blend films are shown in Fig. 3.34. The absorption peaks around 1640 cm^{-1} and 1560 cm^{-1} are attributed to asymmetric stretching and bending of acetamido groups, respectively. The change in the characteristic shape of the chitosan spectrum as well as shifting of peak to a lower frequency range due to hydrogen bonding between –OH of PVA and –OH or NH_2 of chitosan were observed in the blend films. To determine the blending ratio, a base line was drawn with reference to –CH stretching (by PVA) around 2900 cm^{-1} and the ratio of absorption was made with respect to 1550 cm^{-1}. A graph was plotted

between chitosan-PVA concentration v/s 1550/2900 cm^{-1} ratios (Fig.3.35), whose regression coefficient was found to be 0.94. It is reported that water molecule acts as a bridge by intercalating in the polymeric network.

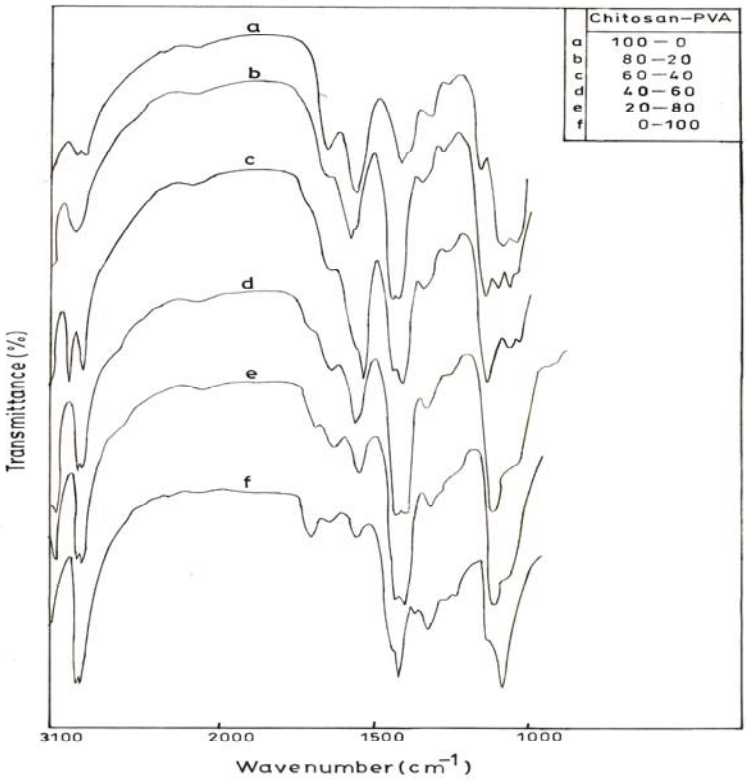


Fig. 3.34. FTIR spectra of Chitosan-PVA blend films

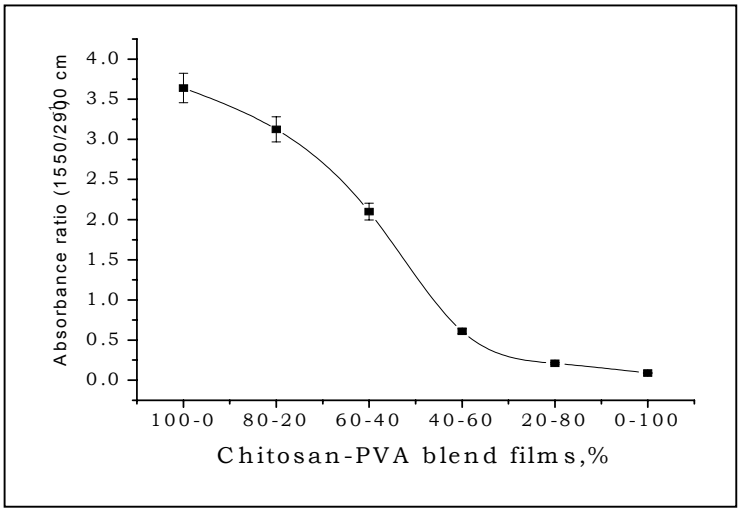


Fig. 3.35. Ratio of absorbance at (1550/2900) cm^{-1} of Chitosan-PVA blend films

DSC of Chitosan/PVA blend films

Thermograms of chitosan-PVA blend films are shown in Fig.3.36. An endotherm around 140 to 160° C, observed in blend film was due to water holding capacity. For native chitosan film the endotherm peak was at 140° C and for native PVA film it was at 159° C. The blend film at 80-20 ratio showed enormous retainment of water (~2 fold) compared to that of native chitosan and 3 fold more compared to PVA films, i.e. –431.11 J/g, -286.11 J/g and –163.78 J/g respectively (Table 3.7). Blending with PVA results in another endotherm around 215° C, solely due to the additional melting endotherm peak of PVA, where ΔH values increased from –7.11 to –34.02 J/g (100% PVA). The exothermic peak of chitosan at around 300°C was diminished as PVA concentration increased due to overlapping of PVA endotherm. In 80–20 blend the heat capacity decreased from 166.54 to 41.16 J/g, due to a negative effect of melting endothermic peak of PVA. Upon blending the same was increased, from 305 (-78.758 J/g) to 316.85° C (-480.42 J/g) with a huge increase in ΔH values.

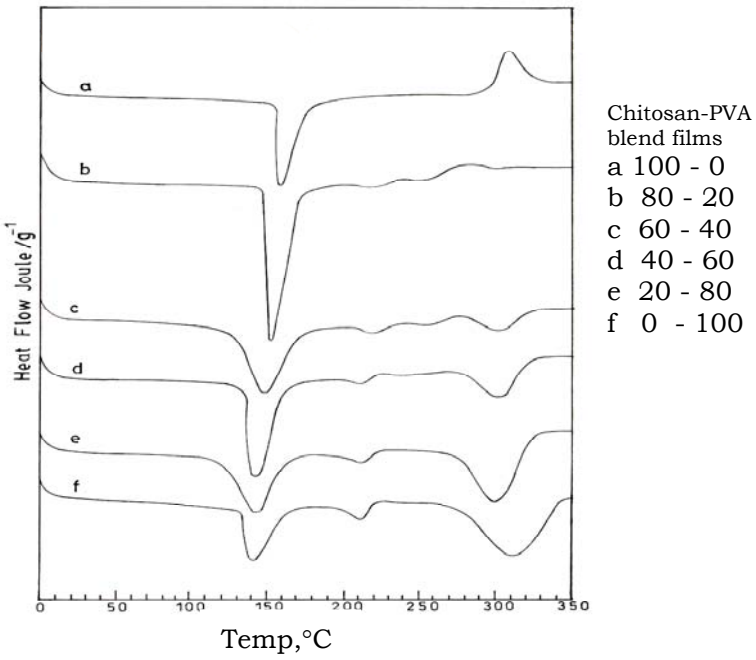


Fig.3.36. DSC thermogram of Chitosan-PVA blend films

Table 3.7. Melting and heat of fusion of Chitosan-PVA blend films measured by DSC

Chitosan %	PVA %	Endotherm		Endotherm		Endotherm		Exotherm	
		T_p	ΔH	ΔH	T_p	ΔH	T_p	ΔH	T_p
100	0	140.43	-286.11					166.54	311
80	20	147.52	-431.11	-7.11	211.26	-	-	41.16	286.36
60	40	143.30	-329.97	-16.07	221.72	-78.58	305.50	52.05	280.92
40	60	147.32	-275.74	-14.87	214.50	-149.16	303.04		
20	80	153.04	-272.66	-28.32	212.76	-317.14	298.41		
0	100	159.21	-163.78	-34.02	216.24	-480.42	316.85	-	-

Wide angle X- ray diffraction of chitosan-PVA blend films

Wide-angle diffractogram (Fig.3.37) of blend films showed that 15° peak intensity got increased may be due to rapid evaporation of water during the drying process. Typical 10° and 20° peaks were found around 11.92° and 21.28°, respectively. Another peak around 23.28° was found due to drying of chitosan acetate salts. The blend film with the ratio 20-80 (chitosan-PVA) showed a drastic decrease in peak intensity of 15°, probably due to the influence of PVA in avoiding the formation of anhydrous crystals in between polymeric chains. The crystallinity of chitosan acetate salt was reduced, as the 23° peak was totally diminished. The intensity of peak around 19° was increased as the concentration of PVA increased. The results showed a perfect blending of PVA with chitosan. It is likely that increase in moisture content of blend film increases crystallinity due to some hydrogen bond formation in the films.

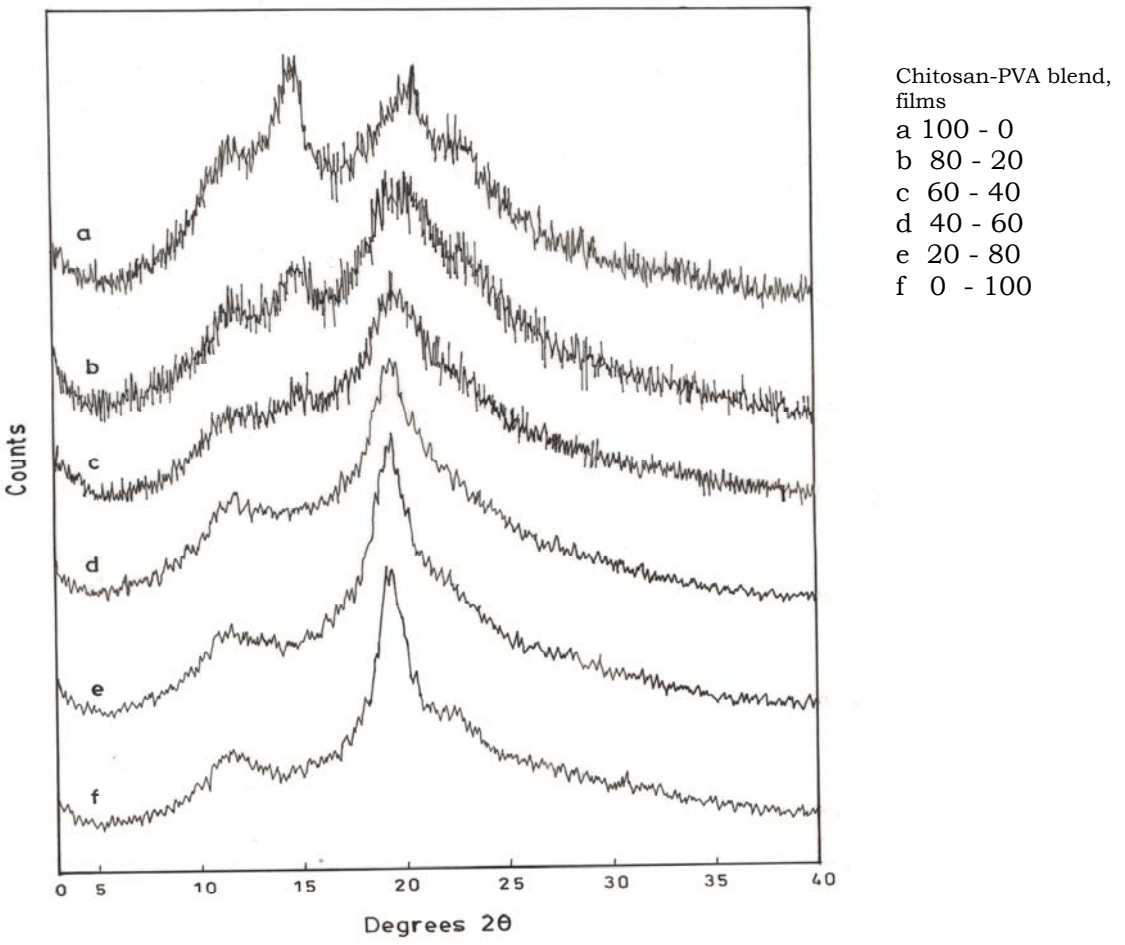


Fig.3.37. X-ray diffraction pattern of Chitosan-PVA blend films

Sorption studies of blend films

The relationship between a_w and moisture content (at constant temperature) is described by moisture isotherm. Moisture content of the film increases at elevated water activity (a_w). The time to reach equilibrium moisture content (EMC) was about 25-30 days at lower humidity and 15-20 days at higher humidities. The sorption isotherm curves for EMC (db) obtained form different concentrations of chitosan-plasticizer blends, shown in Figs.3.38-3.40. At lower a_w the slope of the curve was less, with increase in a_w the slope increased rapidly. Considering the individual blend films, some mould growth was observed by visual inspection at edges and surfaces

at the end of the storage period in native chitosan, and plasticizer blend films, but in fatty acid blend films, less number of colonies were observed may be due to less moisture holding capacity of such films. In chitosan-PVA blend films of ratio 80-20 and 60-40 least number of colonies were observed.

Experimental data for moisture adsorption at 25^0 C for polyols (Fig. 3.38), fatty acids (Fig. 3.39) and chitosan-PVA blend films (Fig.3.40) revealed sigmoid shape curves for all. The EMC of glycerol and sorbitol blend films showed logarithmic increase at above 0.6 a_w and reached to highest moisture content of 45.2% and 43.6% at 0.9 a_w, whereas PEG blend films did not show much increase in moisture content, at 0.4 a_w, the uptake of moisture was more compared to other films, and this showed a linear increasing trend up to 0.7 a_w, but later no exponential increase was observed. Addition of PVA to chitosan films showed increase in moisture content at higher a_w. Fatty acid blend films showed a linear increase in moisture content as a_w increased and showed lower moisture holding capacity compared to other blend films (15.45%). No significant differences were observed between the two fatty acids.

Moisture isotherm equations

Eight moisture isotherm mathematical models were fitted to the moisture sorption data of all blend films for the whole range of a_w. The constants for respective model and root mean square error (RMSE) values were tabulated in Tables 3.8, 3.9 and 3.10, respectively for polyols, fatty acids, and synthetic water-soluble polymer blend films.

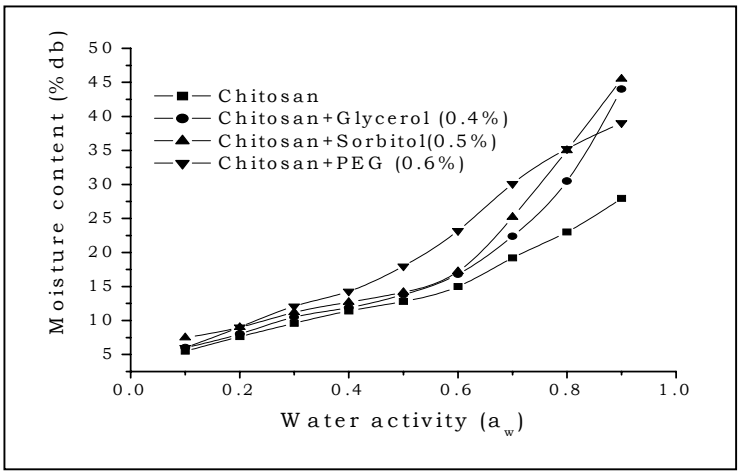


Fig. 3.38. Sorption isotherm of chitosan-Polyol blend films

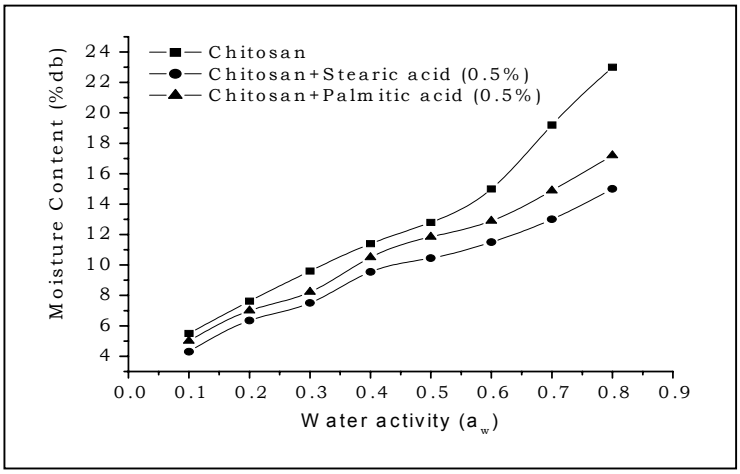


Fig. 3.39. Sorption isotherm of chitosan-fatty acid blend films

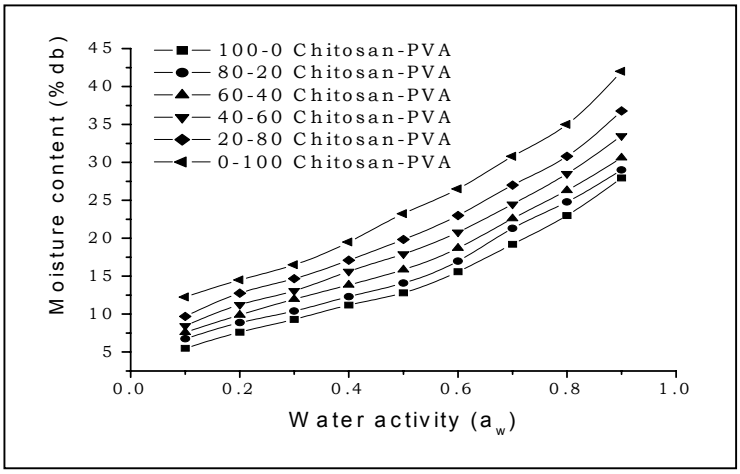


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Fig.3.40. Sorption isotherm of Chitosan-PVA blend films

Table 3.8. Sorption isotherm model constants and co-efficient of regression (R^2) values for Chitosan-Polyol blend films

Model isotherm	Range of water activity (a_w)	Blend ratio, %	Constants of linear fitting			R^2	RMSE
			M_m	C			
BET	0.1-0.5	Chitosan	6.85	33.159		0.99	5.17
		Gly (0.4)	7.32	33.05		0.99	4.96
		Sor (0.5)	7.17	45.00		0.99	9.55
		PEG (0.6)	9.81	11.50		0.99	1.98
			M_o	G	K		
GAB	0.1-0.9	Chitosan	8.83	15.48	0.78	0.99	2.38
		Gly (0.4)	8.15	21.01	0.91	0.99	2.75
		Sor (0.5)	8.18	45.28	0.94	0.99	6.16
		PEG (0.6)	14.1	6.87	0.98	0.99	6.36
			a	b			
Caurie	0.1-0.9	Chitosan	1.61	1.91		0.99	5.11
		Gly (0.4)	2.30	1.58		0.98	4.63
		Sor (0.5)	2.20	1.71		0.98	12.39
		PEG (0.6)	2.31	1.71		0.97	9.53
			a	r			
Halsey	0.1-0.9	Chitosan	0.2174	-1.837		0.97	7.33
		Gly (0.4)	0.4647	-1.541		0.99	4.51
		Sor (0.5)	0.6361	-1.596		0.98	7.19
		PEG (0.6)	0.2846	-1.363		0.82	27.12
			M_b	M_a			
Smith	0.1-0.9	Chitosan	5.60	-10.24		0.99	3.92
		Gly (0.4)	3.26	-16.98		0.99	7.06
		Sor (0.5)	3.95	-17.90		0.98	9.31
		PEG (0.6)	6.69	-16.01		0.95	7.67
			a	n			
Oswin	0.1-0.9	Chitosan	13.04	0.38		0.99	3.72
		Gly (0.4)	15.15	0.46		0.98	6.14
		Sor (0.5)	16.56	0.44		0.95	10.88
		PEG (0.6)	17.60	0.46		0.97	10.05
			K_1	K_2			
Bradley	0.1-0.9	Chitosan	0.88	4.27		0.99	6.02
		Gly (0.4)	0.92	2.53		0.93	15.31
		Sor (0.5)	0.93	2.60		0.95	17.66
		PEG (0.6)	0.92	3.39		0.98	6.19
			A	B			
Harkins-Jura	0.1-0.6	Chitosan	68.73	-0.23		0.95	9.02
		Gly (0.4)	80	-0.24		0.96	7.17
		Sor (0.5)	124.07	-0.11		0.98	2.59
		PEG (0.6)	77.96	-0.37		0.90	13.3

Table 3.9 Sorption isotherm model constants and co-efficient of regression (R^2) values for Chitosan-Fatty acid blend films

Model isotherm	Range of water activity (a_w)	Blend ratio (%)	Constants of linear fitting			R^2	RMSE
			M m		C		
BET	0.1-0.5	Chitosan	6.85		33.159	0.99	5.17
		Ste (0.5%)	5.66		27.169	0.99	5.78
		Pal (0.5%)	6.31		25.54	0.99	3.55
			Mo	G	K		
GAB	0.1-0.9	Chitosan	8.83	15.48	0.78	0.99	2.38
		Ste (0.5%)	8.44	14.51	0.60	0.98	2.30
		Pal (0.5%)	8.92	14.96	0.64	0.99	3.30
			a		b		
Caurie	0.1-0.9	Chitosan	1.61		1.91	0.99	5.11
		Ste (0.5%)	1.57		1.49	0.96	10.63
		Pal (0.5%)	1.60		1.62	0.97	6.40
			a		r		
Halsey	0.1-0.9	Chitosan	0.2174		-1.837	0.97	7.33
		Ste (0.5%)	0.1836		-2.128	0.90	11.07
		Pal (0.5%)	-0.050		-2.115	0.95	9.49
			M b		M a		
Smith	0.1-0.9	Chitosan	5.60		-10.24	0.99	3.92
		Ste (0.5%)	5.60		-5.64	0.94	7.02
		Pal (0.5%)	6.08		-6.68	0.95	6.98
			a		n		
Oswin	0.1-0.9	Chitosan	13.04		0.38	0.99	3.72
		Ste (0.5%)	9.748		0.31	0.98	5.91
		Pal (0.5%)	10.98		0.32	0.98	5.51
			K_1		K_2		
Bradley	0.1-0.9	Chitosan	0.88		4.27	0.99	6.02
		Ste (0.5%)	0.79		7.26	0.99	3.73
		Pal (0.5%)	0.82		6.62	0.99	3.74
			A		B		
Harkins -Jura	0.1-0.6	Chitosan	68.73		-0.23	0.95	9.02
		Ste (0.5%)	42.62		-0.23	0.92	11.98
		Pal (0.5%)	58.26		-0.18	0.94	9.75

Table. 3.10. Sorption isotherm model constants and co-efficient of regression (R^2) values for Chitosan-PVA blend films

Model isotherm	Range of water activity (a_w)	Blend ratio, % (PVA-Chitosan)	Constants of linear fitting			R^2	RMSE
			M m	C			
BET	0.1-0.5	0-100	6.78	32.09		0.99	3.97
		20-80	7.32	80.41		0.99	4.21
		40-60	8.23	81.00		0.97	4.60
		60-40	9.32	67.06		0.99	4.04
		80-20	9.58	149.14		0.99	7.07
		100-0	11.74	213.00		0.99	1.07
			Mo	G	K		
GAB	0.1-0.9	0-100	8.83	15.48	0.78	0.99	2.38
		20-80	9.71	18.96	0.76	0.99	3.52
		40-60	11.21	19.16	0.73	0.98	2.78
		60-40	12.69	19.46	0.71	0.98	2.79
		80-20	13.87	22.12	0.71	0.99	1.87
		100-0	15.76	25.59	0.71	0.99	3.87
			a	b			
Caurie	0.1-0.9	0-100	1.61	1.91		0.99	5.11
		20-80	1.78	5.96		0.99	3.02
		40-60	1.68	6.89		0.97	3.30
		60-40	1.64	7.80		0.99	3.58
		80-20	1.58	8.85		0.99	3.57
		100-0	1.52	10.61		0.99	1.30
			a	r			
Halsey	0.1-0.9	0-100	1.243	-1.837		0.97	7.33
		20-80	1.355	-1.984		0.96	10.89
		40-60	1.283	-2.094		0.96	9.17
		60-40	1.248	-2.143		0.96	9.02
		80-20	1.328	-2.225		0.97	9.45
		100-0	1.432	-2.3289		0.96	12.44
			M_b	M_a			
Smith	0.1-0.9	0-100	5.62	-10.34		0.99	3.92
		20-80	6.87	-10.43		0.97	7.38
		40-60	8.13	-10.63		0.99	8.56
		60-40	9.26	-11.40		0.97	8.96
		80-20	10.50	-12.27		0.98	8.23
		100-	12.35	-13.74		0.99	6.28
			a	n			
Oswin	0.1-0.9	0-100	13.04	0.38		0.99	3.72
		20-80	14.35	0.35		0.99	4.60
		40-60	16.95	0.33		0.99	3.9
		60-40	17.61	0.32		0.99	3.59
		80-20	19.45	0.31		0.99	3.32
		100-0	22.71	0.30		0.97	4.03

			K_1	K_2		
Bradley	0.1-.9	0-100	0.88	4.27	0.99	6.02
		20-80	0.88	4.87	0.99	6.37
		40-60	0.88	5.63	0.99	3.64
		60-40	0.89	5.95	0.99	2.77
		80-20	0.90	6.41	0.99	5.24
		100-0	0.91	6.78	0.99	6.12
			A	B		
Harkins Jura	0.1-0.6	0-100	68.73	-0.229	0.95	9.02
		20-80	106.45	-0.116	0.98	4.24
		40-60	134.48	-0.115	0.97	5.95
		60-40	169.19	-0.106	0.97	5.79
		80-20	224.58	-0.064	0.98	4.82
		100-0	347.98	-0.025	0.99	2.40

The applicability of BET model is limited to a lower range of a_w (0.1-0.4), whereas PEG blend films showed a goodness of fit above this range. The monolayer (Mm) and energy constant (C) of blend films varied from 6.85-9.81 and Constant (C) from 11.50-45. PEG blend data suits very well in that range compared to those of other blend films. Palmitic acid blend films showed a better fit with RMSE value of 3.55 compared to stearic acid blend films (RMSE 5.78). The native PVA showed a higher Mm (11.74) and C (213.00) value and its blend film showed a better fit compared to all other films with RMSE value of 1.07. Baldevraj *et al.* (2002) have studied the sorption characteristics of starch-LDPE and PVA blend films and determined the various constants.

The GAB model is the most popular model in the area food technology. Sorption data fits extremely well for many food materials over a wide range of a_w (Bizot, 1984). But so far only limited studies have been carried out with blend films. GAB model is a choice for PVA and fatty acid blend chitosan films with RMSE values <3. In some plasticizer blend films the RMSE values were between 3 and 5, an R^2 value of 0.99, Mo value in the range 8 to 15.76, G value in the range 14.60 to 25.6, and K value in the range 0.59 to 0.912 (for Chitosan blend with PVA/fatty acid). The Chitosan-PVA blend films, showed lower RMSE values compared to those of native PVA and chitosan films. Chirife *et al.* (1992) reported that the constants K in GAB sorption

model varies from nearly unity to as low as 0.56 for large variety of food constituents and for proteins in the range of 0.82 to 0.88. Gennadios and Weller (1994) reported that GAB model was a better fit when applied over the a_w range from 0.33 to 0.84 for protein films with high values of K. Lemauro *et al.* (1985 a, b) found that the GAB model gave a very good fit (P<5) for over 50% of 75 food isotherms for fruits vegetables, and over 75% of 88 isotherms for coffee, tea, nuts etc.

Bradley model can be widely used for a wide range of a_w (0.05 to 0.95) including sorption of water in proteins (Hoover and Mellon, 1950). The constants (K_1 and K_2) were determined by linear fitting of the equation Table 3.1**,** where K_2 is a function of sorptive polar groups and K_1 is a function of the dipole moment of the sorbed vapor, and both these constants were found to be temperature dependent. K_1 showed not much significant variation, it varied from 0.8 to 0.9, but K_2 varied from 2.53 to 17.67 for all blend films. Baldevraj *et al.* (2002) reported that the Bradley sorption model fits very well in the a_w range between 0.4 to 0.9. Walker *et al.* (1973) reported that for peanut protein films. Bradley model would fit for a_w 0.3 to 0.9. In the present study, the Bradley sorption model was found to fit very well for the whole range of a_w except for glycerol and sorbitol blend films, where the fit was possible between a_w 0.4-0.9.

Smith model has also been shown to fit water sorption isotherms of various biopolymers (Smith, 1947). A good fit was documented for adsorption and desorption isotherms of Virgina-type peanuts above a_w of 0.3 (Young, 1976). Pixton and Howe (1983) reported that the Smith model gave a satisfactory fit for water sorption curves of several food commodities including food bean, dried peas, dried figs, etc. The model showed a perfect fit for native chitosan film, compared to all other blend films with RMSE value of 3.92. Protein films showed goodness of fit in the range of 0.53-0.84 a_w (Gennadios and weller,1994), whereas for above this range the data showed very poor fit, as also observed in the present study.

The Oswin model was found very suitable to describe sorption isotherms of proteins and starchy foods (Boquet *et al.*, 1978). Lomauro *et al.* (1985 a,b) concluded that this model fitted sorption data for a considerable number of nuts, spices, coffee, etc,. In the present study the native chitosan film showed a perfect fit in the whole range of a_w with RMSE value of 3.72 with constants n and A of 0.38 and 13.03, respectively. For the plasticizer blend films these constants varied from 0.44-0.46 and 15.15 to 17.6 and RMSE of 6.14 to 10.89 respectively, whereas for PVA-chitosan blend films the RMSE values were in the range 3.32-4.60, showing the perfect fitting of this model in whole range of a_w. The fatty acid blend films showed RMSE of 5.91 and 5.51 respectively for stearic and palmitic acid. There was no considerable difference observed in the constants (n, A) values for the two fatty acids: n= 0.31 and 0.32 and A=9.75 and 10.98 for stearic and palmitic acid blend films, respectively.

Use of Halsey model is recommended for meats, milk products, and vegetables (Boquet *et al.*, 1978). According to Lomauro *et al.* (1985 a,b), this model was successfully applied for water sorption data of several nuts and oil seeds. Iglesias *et al.* (1975) and Chirife and Iglesias (1978) found that Halsey's model is useful to describe reasonably well the sorption of dried figs, apricots and raisins and the total number of experimental isotherms to which they applied satisfactorily the Halsey's model amounted to 220. When this model was used to fit sorption data of chitosan blend films it showed a poor fit for a_w 0.1 to 0.8. Nevertheless, this model gave excellent fit for sorbitol blend films (RMSE 4.04), whereas for all other films with RMSE values in the range 6.12 to 12.06, it showed a poor fit especially for PEG films with RMSE value of 27.12.

Caurie model, purely a mathematical equation valid for a_w 0.0 to 0.85, fits very well to PVA-chitosan blend films. Native chitosan and PVA showed RMSE values of 5.11 and 1.30 respectively, whereas all their blend films RMSE values were ranged between 3.03-3.58. The regression R^2 of native

and blend films was 0.99. In plasticizer blend films only glycerol blend film showed a good fit (RMSE, 4.63), whereas all other blends with high RMSE values showed a poor fitness of the model. In fatty acid blend films stearic acid films showed high RMSE (10.63) compared to palmitic acid blend films (RMSE, 6.40). With the addition of PVA the constant 'a' increased, and 'n' decreased. Whereas in plasticizer and fatty acid blend films no such differences were observed in these constants.

Harkins-Jura sorption isotherm model is restricted to regions in which the adsorbed molecules form a condensed film layer (Labuza, 1968). The equation usually does not holds good above a_w of 0.6, as also seen in the present study, where the model is fit for a_w in the range of 0.1 to 0.6. The model showed goodness of fit for native PVA and sorbitol blend films (with low RMSE value 2.40 and 2.59). For all other films the RMSE values were above 5, and showed a poor fit of the model.

Overall, the sorption analysis of different models showed extremely good to very good fit as determined by RMSE and R^2 values. The constants derived from different sorption models were useful in the evaluation of the stability of chitosan-based packaging films. The applicability of water activity values will throw valuable information on the durability of packaging material for specific end uses. Also the constants derived from the respective models could be utilized to predict the EMC values, in comparison with the experimental values. It can be observed from Figs.3.41-3.43, that all the models could successfully predict the EMC values at all combinations of chitosan-PVA. However, the GAB model had the lowest RMSE and highest R^2 values, indicating it to be the best model. Linear models with high R^2 and low RMSE are considered to be statistically acceptable.

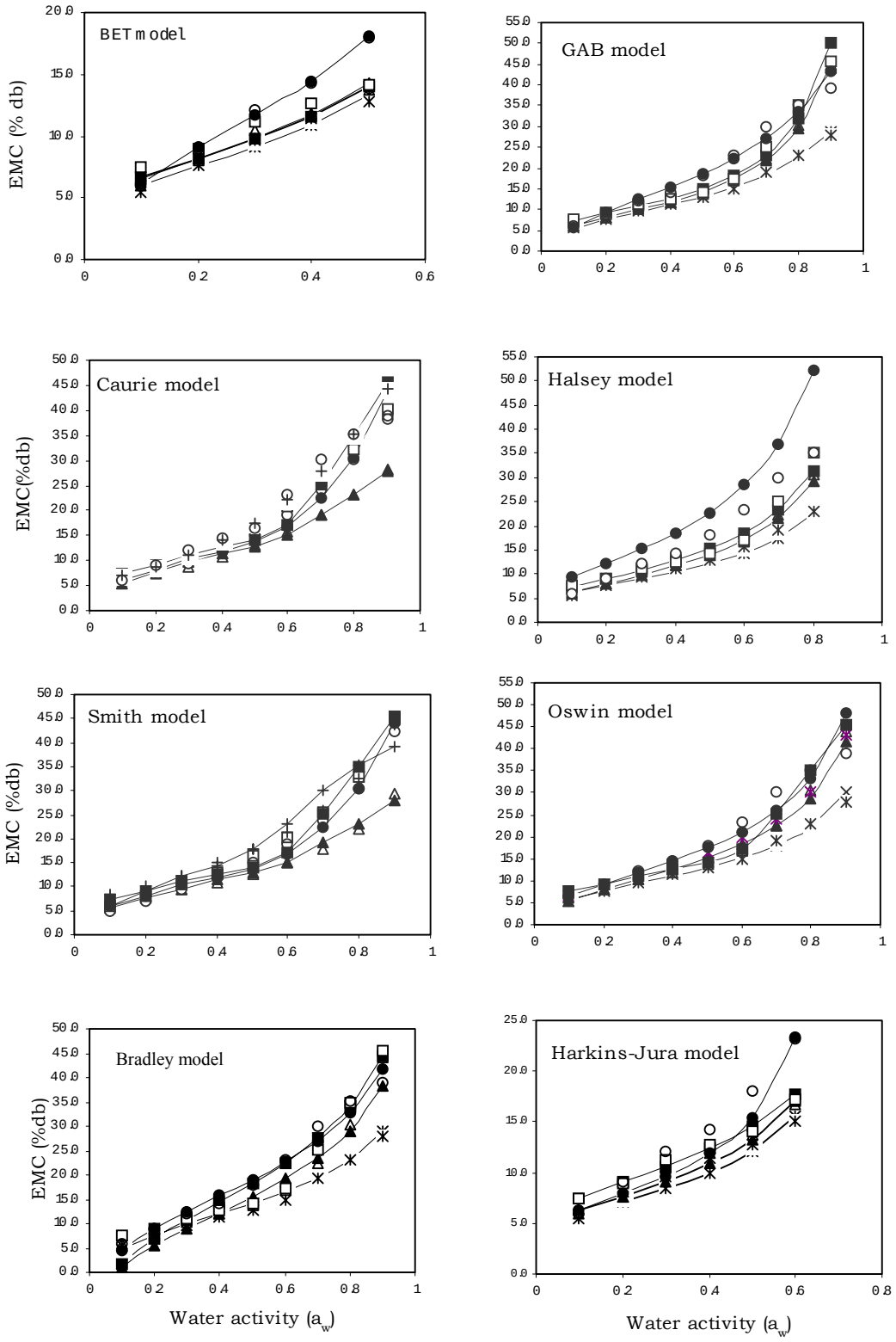

Fig.3.41. Sorption isotherm of Chitosan-Polyol blend films with various sorption models obtained through experimental (lines) and predicted (symbols)

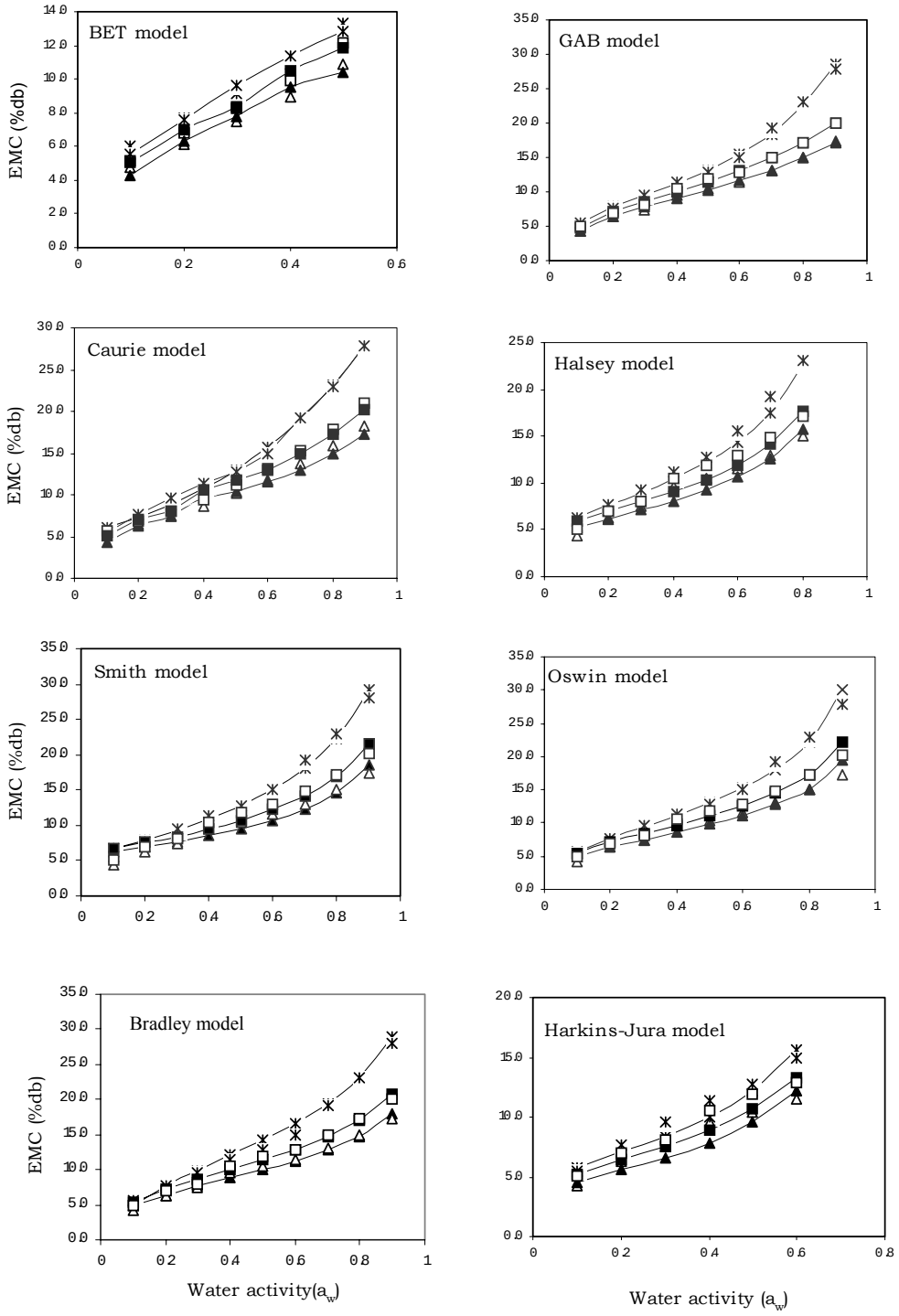

Fig.3.42. Sorption isotherm of Chitosan-Fatty acid blend films with various sorption models obtained through experimental (lines) and predicted (symbols)

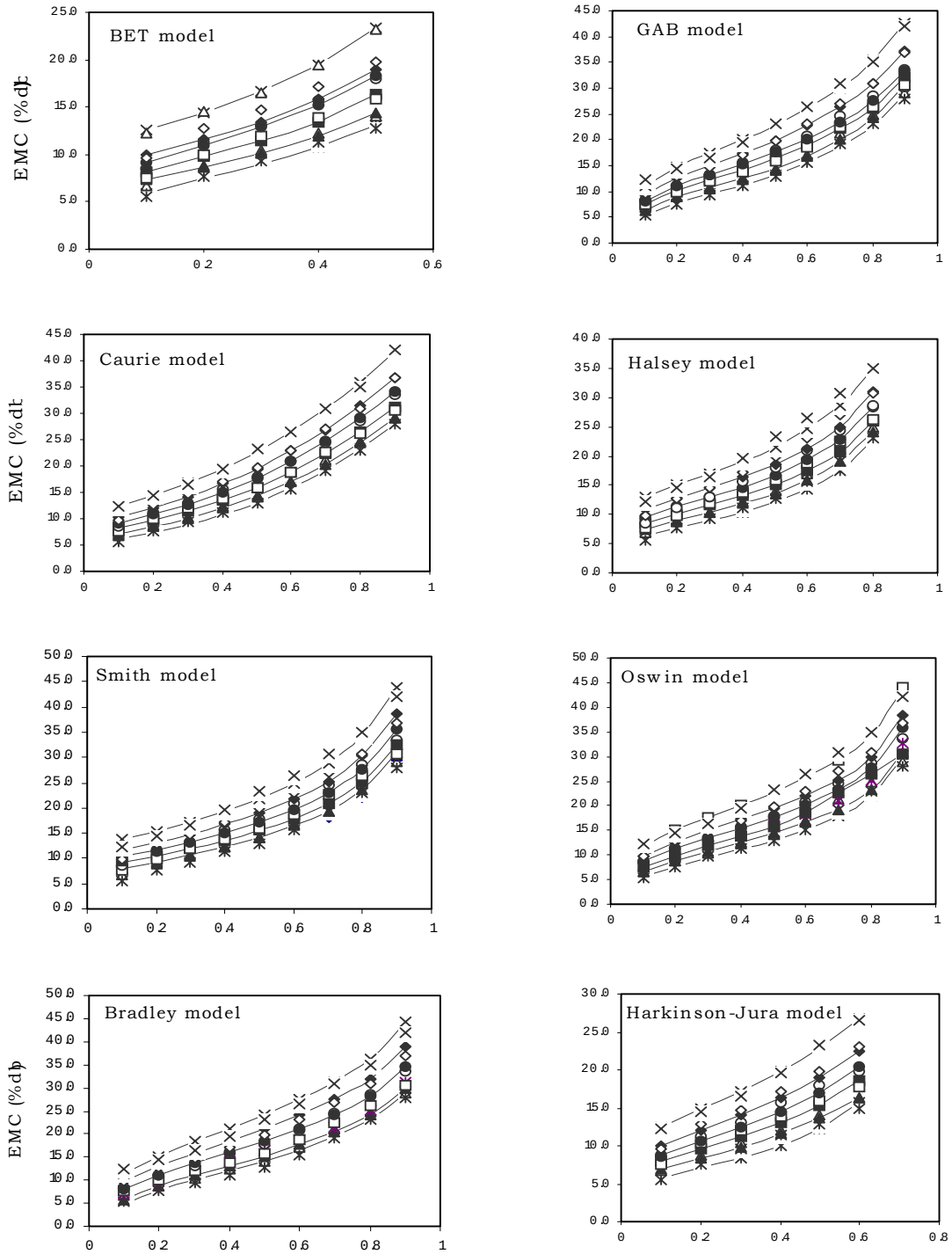

Fig.3.43. Sorption isotherm of Chitosan-PVA blend films with various sorption models obtained through experimental (lines) and predicted (symbols)

Biodegradation of chitosan films

Biodegradation usually takes place by microorganisms (in the soil or environment), which will utilize the films as a sole carbon source by degrading their polymeric structure. SEM of native chitosan films showed a smooth surface initially and the films were fragmented into pieces in a short period of time, when in soil (5-6 days) and releasing mono/oligomers. This may be attributed to their hydrophilic nature, which make the polymer chains to become weaker and fragile and thus allowing soil microorganisms to attach and attack. During degradation visual fungal colonies were observed on the edges and surface of films with in 3-4 days.

Polyols and fatty acid blend chitosan films were found biodegraded in 5-7 days and 12-15 days respectively. The polyol blend films were degraded similar to native chitosan films, probably due to improved flexibility of the films because of weakening of the polymer chains (Fig.3.44), whereas in fatty acid blend films, because of their higher hydrophobicity it takes more time (12-15 days) to degrade.

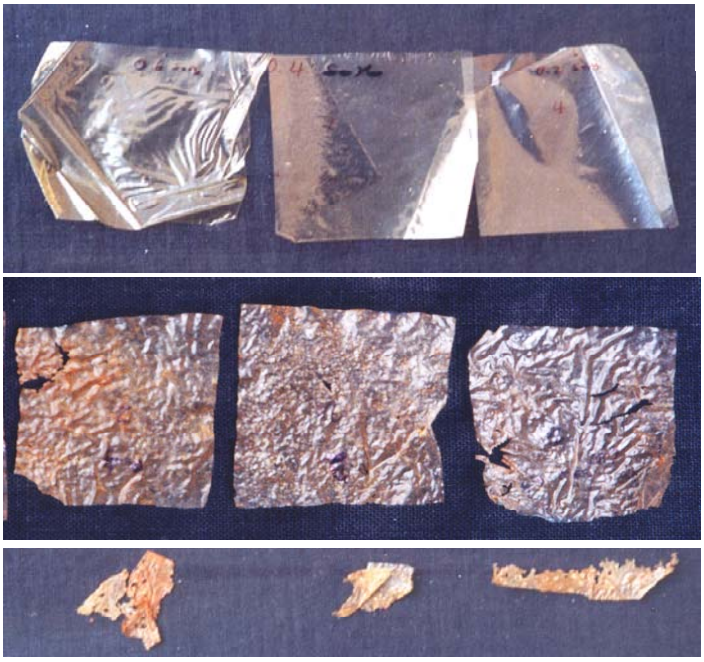

Fig. 3.44. Biodegradation of polyol blend films

The chitosan-PVA blend films upon SEM observation (Fig.3.45) showed smooth surface, without any visible degradative changes. As the

concentration of chitosan increased the degradability was more. At 20-80 PVA-chitosan ratio, biodegradability was attained after 30 days, whereas at higher PVA concentration a sieve like structure was observed after 100 days (Fig.3.45). Only a partial degradation of 60-40 PVA-chitosan was observed after 100 days (Fig.3.45). More than 80% of PVA did not show any type of degradation even after 120 days. Even though PVA is a water-soluble polymer, its biodegradation was hindered.

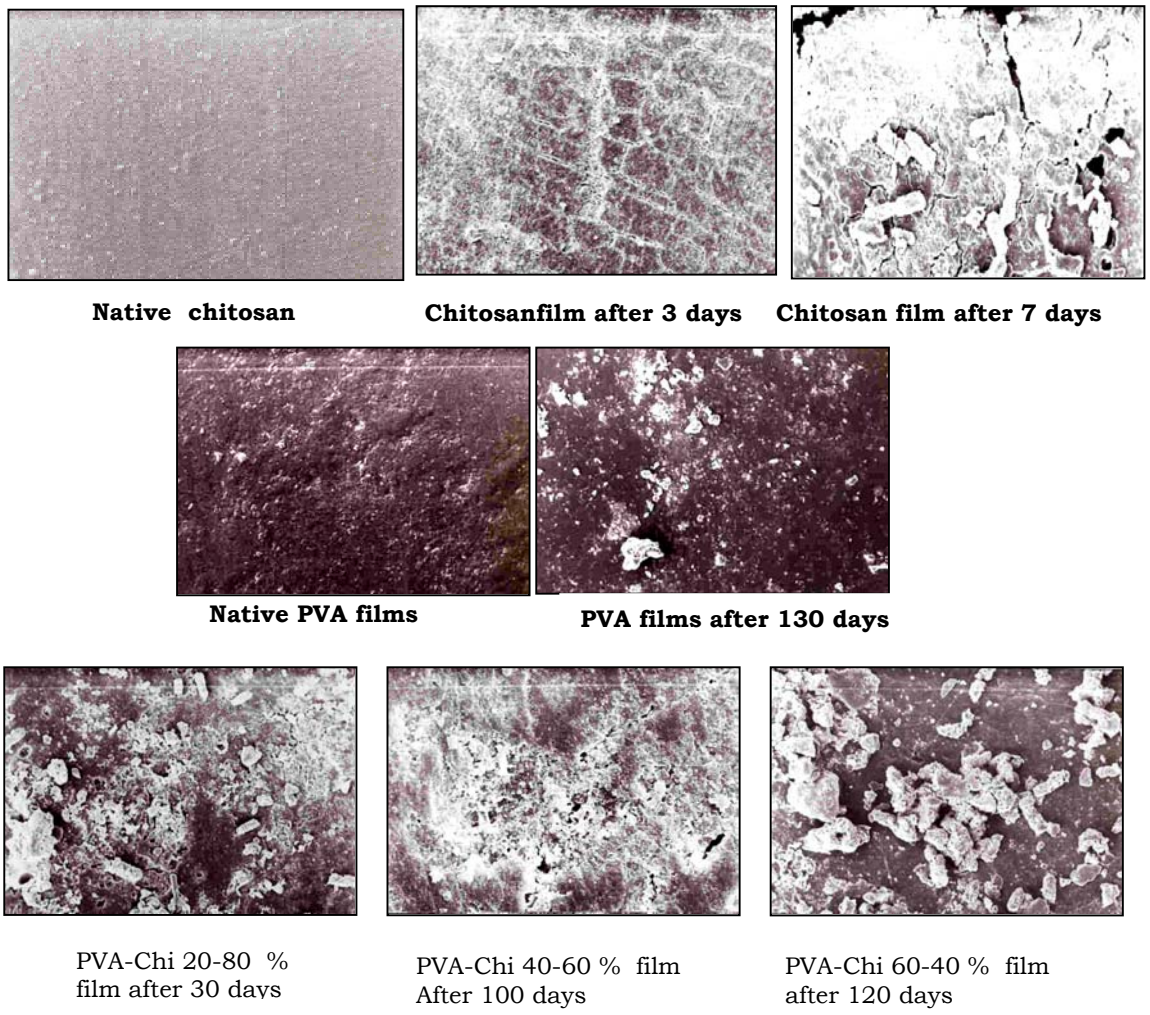

Fig. 3.45. Biodegradation of chitosan-PVA blend films

Conclusions

Modifying the chitosan film with polyols, fatty acids and a water-soluble polymer, PVA considerably affected the properties of the film. Fatty acid blend films showed decreases in elongation property. The TS of chitosan –PVA blend films showed both increase and decreasing trends. The oxygen barrier properties decreased with the addition of PVA. The FTIR results showed change in characteristic shape as well as shifting of peaks in blend films. DSC also showed altered thermograms. Chitosan –glycerol blend films showed an additional endotherm peak. Chitosan-PVA blend film showed the characteristic peak of chitosan at 300 °C diminishing and appearance of an additional peak at 215 °C. X-ray pattern showed shifting of diffraction peaks in the blend films. Moisture sorption isotherms showed sigmoid pattern, with GAB model showing a better fit compared to other models over a wide range of water activity values. Polyol and fatty acid blend films showed faster bio-degradation.

Introduction

A range of techniques is used to preserve post harvest quality of fruits, vegetables, and other perishable produce. Refrigeration is a common preservation technique used but, in some instances, low temperature alone may be insufficient to retard ripening of fruit and prevent detrimental quality changes. Moreover, low temperature for prolonged period may lead to physiological changes (Smith *et al.*, 1987b). Kidd and West's (1927) pioneering work lead to the development of controlled atmosphere (CA) storage technique to extend the shelflife of fruits and vegetables. Here the concentrations of CO_2 and O_2 are controlled at optimal levels for each cultivar, facilitated by the recent developments of automatic control systems. However, both refrigeration and CA techniques are more expensive, requiring large capital outlay for installation and maintenance, and also require high-energy inputs, especially for cooling. Moreover, it is not practical to use these techniques for small quantities of produce or individual fruits. Thus, once the produce is harvested and removed from storage, it is subjected to ambient conditions during marketing, which often lead to rapid quality deterioration (Smith *et al.*, 1987a).

With increasing consumer awareness of quality in fresh produce, methods have been sought to create microclimates surrounding small quantities of produce that would continue to mimic the beneficial effect of CA storage into and through the marketing chain, ideally without the use of refrigeration. The use of artificial barriers to gaseous diffusion may provide the means of achieving quality maintenance and reduction or elimination of physiological and pathological disorders (Smith *et al.*, 1987a).

Successful CA application requires that the atmosphere surrounding fruit contain elevated CO_2 and/or reduced O_2 concentrations, and it varies from commodity to commodity. The most marked effects of reduced O_2 concentration at storage temperatures are seen below 4%, but below 0.1%

problems of physiological disorders and alcohol formation may occur (Knee, 1980; North and Cockburn, 1976).

In any method at a given temperature, increased resistance to diffusion of reduced CO_2 and O_2, concomitantly reduces the respiration rate, which leads to establishment of new equilibrium concentrations of gases surrounding the fruits. The relative importance of altered O_2 and CO_2, and ethylene concentration and water vapor is unknown, and there may be direct effects of barrier application on the quality, that may not be mediated through alteration of gas concentrations (Smith *et al.*, 1987b; Smith and Stow, 1984).

Artificial barriers to diffusion can be achieved by coating and packaging films. The term "coating" as used here refers to a thin layer of a foreign material applied to the surface of fruits/ vegetables, as an additional covering over the natural protective cover. The coating may be applied by dipping or drenching or spraying, or for experimental purpose by hand with a brush. The coating material may be oil, wax, polysaccharide, protein, or formulations, etc,. Much of the earlier work used wax and oil based materials and problems observed were delay in firmness and colour development of apple with off-flavor development due to over modification of CO_2 and O_2 levels around the fruit (Chu, 1986; Eaves, 1960; Elson *et al.*, 1985). But the problem is probably due to non-uniformity of coating leading to progression of anaerobiosis, and spoilage of fruits / vegetables (Dhalla and Hanson, 1998). But recent development claims the use of base materials such as water-soluble carbohydrates like sucrose esters, proteins, etc., which have been applied to apple, pear, banana and mango. Chitosan has also been used to extend the shelflife of capsicum, banana, strawberries and tomatoes (Kittur *et al.*, 2001;Ghaouth *et al.*, 1991,1992).

The modification of internal atmosphere concentrations by use of coatings can increase disorder associated with high CO_2 or low O_2

concentrations, such as core flush (Smith and Stow, 1984), flesh browning and break down (Trout *et al.*, 1953) and accumulation of ethanol and alcoholic off-flavors (Cuquoerella *et al.*, 1981; Trout *et al.*, 1953).

Films are extruded plastic materials that are used to surround the produce as shrink or stretch wraps, or as seated loose covers creating modified atmosphere (Marcellin, 1974). Films have been employed to restrict water loss in storage for many years, but their use in modified atmosphere packs has been relatively limited. The equilibrated atmosphere achieved in the presence of an artificial diffusion barrier primarily depends on the permeability of the films and respiration rate of produce enclosed. Much of film packaging is done using synthetic petroleum based films, which have both water barrier and gas permeable properties.

Mango is an important commercial, seasonal fruit of India and having excellent export potential. Considerable research has been carried out to improve its post harvest handling and to extend the storage life [Salunke and Desai, 1984; Miller *et al.*, 1986a). Annual world production of mango is around 23 x 10^6 metric tons and India's contribution is 12 x 10^6 metric tons (FAO, 1999). Low temperature storage is the most commonly adopted method to extend the shelflife of mangoes, although spoilage losses due to chilling injury have been reported [Miller *et al.*, 1986b; Lakshminarayana and Subramanyam 1970). Low temperature with modified atmosphere or controlled atmosphere packaging using various synthetic plastic films show increased CO_2 and decreased O_2 levels resulting in considerable increase in shelflife (Rodov *et al.*, 1997). Shelflife extension of mango by packing with synthetic film, storing at low temperature and later transferring to ambient temperature showed no significant changes in biochemical and other parameters (Miller *et al.*, 1983). Low O_2 and increased CO_2 levels in modified/controlled atmosphere packaging resulted in reduced ethylene production and respiratory activity, better flavor retention, reduced softening rate and slower green colour loss (Gonzalez-Aguilar *et al.*, 1997). Shelflife of

mangoes could also be extended by coating the fruits with polysaccharide formulations (Baldwin *et al.*, 1999; Kolekar *et al.*, 1992). In coated fruits the respiratory rate could not be controlled, which led to anaerobiosis and significant loss of sensory quality (Kolekar *et al.*, 1992). A few composite coating formulations, prepared from chitosan and its N, O-carboxymethyl derivatives, have been used for considerable shelflife extension of banana and mango fruits (Kittur *et al.*, 2001). Fruits and vegetables continue to actively metabolize during post harvest phases, which include harvesting at optimum maturity, minimizing mechanical injuries, optimum temperature and relative humidity during transportation and marketing, and all these influencing the quality and shelflife of such commodities. Yet another factor to control respiration is the modification of surrounding atmosphere.

As for vegetables were concerned, the modification of surrounding environment can be done by individual coating of the vegetables or by sealing in the polymeric films. Wax is extensively used as a coating material, but it enhances the risk of off-flavor development and fermentation due to drastic reduction in gas permeability of the peel (Cuquerella *et al.*, 1981). Use of plastic films for different citrus species (lemons, oranges and grape fruits) has given better responses than waxing in preserving the overall quality, shrinkage, softening, deformation and flavor loss (Agabbio, 1990; D'Aquino *et al.*, 1999). Corn zein coating on tomatoes delayed color change and loss of firmness (Park *et al.*, 1994). Bell pepper individually wrapped in plastic film showed marked reduction in weight loss and softening, which interfered with extension of shelflife (Hughes *et al.*, 1981; Gonzalez and Tizando, 1993). Another limitation of using plastic materials concerns environment, due to non-biodegradability. Replacement of plastic films with edible or biodegradable materials is a desirable eco-friendly approach.

Indigenous milk based products of several types are made in India which are classified broadly into condensed (*khoa, burfi, gulabjamun, peda* etc.), cultured (*dahi, makkhan, lassi, shrikand,* etc.) and acid precipitate

(*panner, sandwich, rasgulla*, etc.) products. Marked variations in their composition, sensory and keeping quality as well as microbiological profile have been observed in the market samples. For dried *khoa*, suitable for use in the preparation of acceptable *peda*, a mean shelflife of 90 days when air packaged and 105 days when N_2 packaged has been reported. The keeping quality of *peda* made from buffalo milk was 6-8 days at 37° C and ~30 days at 5° C. Metatisulfities of sodium and potassium at 1000 ppm levels have been used to increase the shelflife of *khoa* based products. The hygienic conditions under which these products are prepared exert greater influence on their microbiological load and safety for consumption. Average standard plate counts of good, fair and poor *peda* were found to range around 5600, 14000 and 56000 cfu/g, respectively, and the level of counts more than 30,000 is not permissible. Hemavathy and Prabhakara (1973) reported the carbonyl content of stored fresh *khoa and burfi*. The former was rich in short chain and the latter rich in long chain carbonyls. The presence of saturated aldehydeic components led to off-flavour development during extended storage.

Deshmukh *et al.* (1977) studied quality of *khoa* with respect to various levels of total solids and storage temperature a shelflife of 9 days was reported at 30 °C. At 5 °C it had a shelflife of 60 days. There was no significant effect of TS content on bacterial counts and keeping quality was more closely related to yeast and mould counts than bacterial counts. Titratable acidity of fresh *khoa* increased with increasing TS content but had no adverse effect on flavour. The rate of increase in acidity was similar at 30° and 22°C, but considerably slower at 5 °C. *Burfi*, an Indian sweet made from *khoa* containing 90% TS and steamed for 3 min, was as good as that made from *khoa* with 70% TS. Sachdeva and Rajorhia (1982), showed that, the shelflife of *burfi* packaged in parchment paper was about 10 days at 30°C and 50 days at 5°C. The major causes of spoilage were fat oxidation and mould growth, which could partially be alleviated by using 0.015% saffron. Garg and Mandokhot (1987), reported increase in standard plate counts and

in the number of salt tolerant bacteria of freshly prepared as well as stored *burfi*. *S. aureus* multiplied in both *burfi* and *peda*, whereas yeast and moulds were isolated from samples throughout storage. Ghodeker *et al*. (1974) reported *burfi* and *peda* samples becoming sour after 14 days at 30°C, 21 days at 22°C. Increase in acidity due to acid producing bacteria provided favorable environment for growth of yeasts and moulds, which was indicative of unhygienic conditions during manufacture and storage.

Goyal *et al*. (1991), studied *khoa* packed in a 3-ply laminate of paper /aluminium foil / LDPE or 2 ply laminate of MST cellulose and found a shelflife of 10 days at 37°C or 60 days when refrigerated; 4 ply laminate pouches of poly propylene- LDPE / aluminium foil / LDPE extended the shelflife to 14 days at 30°C. Flexible poly films and laminates were recommended for milk-based sweets. *Khoa* was prepared using stainless steel jacketed open kettles, and *burfi* was prepared by adding sugar and 9.15% sorbic acid to *khoa*. Sensory evaluation was carried out after storage with different packaging materials and best results were obtained with *burfi* containing 0.15% sorbic acid and packaged in polycel / PE pouches, (compared to 6-8 days when stored by traditional methods (Ramanna *et al.* 1983). Indiramma *et al.* (2002), studied modified atmospheric packaging for extending the shelflife of *peda*, vacuum packaging was found to retard mould growth, although it adversely affected the texture. The use of free oxygen absorbers was found to be highly beneficial. Naresh *et al.* (2003) studied the quality of *peda* by utilizing microwave processing and MAP techniques. *Peda* when packed in LDPE at different levels of vacuum and stored at 28°C increased keeping quality by 2 folds at room temperature.

None of the above reported information was comprehensive, cost effective, eco-friendly and provided data in a holistic manner. Invariably the packaging was done with LDPE or other synthetic plastic films, which are petroleum based and non-biodegradable. In some, details about the sensory characteristics or on the microbial load of the stored products are not

provided. Use of MAP or vacuum-packaging techniques through claimed to be useful for enhanced shelflife extension, but they are not cost effective and simple. Growing awareness by one and all towards biodegradable and eco-friendly packaging films urges to look for better cost effective and simple alternatives for various food-packaging applications. In this work, preliminary studies were carried out on usability of chitosan films on dairy products.

In bakery products, limited work has been done on using chitosan films or chitosan coated butter paper films on shelflife extension studies. Generally bakery products are also packed in petroleum based plastics films.

Materials and Methods

Mature, green mangoes (*Mangifera indica*, variety Alphanso) were procured from an orchard near Mysore. The fruits were desapped to prevent sap injury and later washed thoroughly with running tap water, then dipped in Carbendazin 50% WP (BASF, Mumbai) (500 ppm) solution for 15 min to prevent fungal attack. The treated fruits were spread on a wire mesh tray and air dried for 30 min. Tomato (*Lycopersicon esculentum*) and bell pepper (*Capsicum annuum*) were procured from local orchards and were treated with Benlate (50 ppm).

LDPE films (100 gauge) and carton boxes were purchased from the local market. Four mangoes were placed in each carton boxes (160 x 220 x 75 mm) (wax coated inside), whose top surface was covered with either chitosan film (MAP 1) or LDPE film (MAP 2). Mangoes kept in carton boxes without any cover served as control. All the boxes were kept at room temperature (27 ± 1°C) and two boxes from each treatment were taken out periodically for analysis.

Physiological loss in weight (PLW)

Initial weight of the fruits was taken and periodical observation on the loss in weight of the stored fruits was recorded. PLW was calculated and expressed as cumulative percentage loss.

Instrumental analyses

Texture analysis

The texture of stored mango was measured by Universal Testing machine (LLOYDS model LR 5K, UK). Penetration test (10 mm depth) was carried out using Magnus Taylor spindle with a speed of 10 mm/min. For compression test, the surface skin was removed and the inside portion of the fruit was cut into a cube (15 x 15 x 15 mm) and the load was applied. Peak load (N) for 50% compression of the specimen was determined.

Firmness of tomatoes and bell pepper fruits was measured by piercing, using Universal Testing machine. Penetration test (10 mm depth) was carried out using 2 mm spindle with a speed of 10 mm/min. For compression of bell pepper, the pods were kept horizontally and the load was applied. Peak load (N) for 50% compression of the specimen was determined. And for shear test same speed was kept but a werener bletzer (WB) needle was used. The load was mentioned in Newton (N).

Colour

Colour of the mango was measured (CR-minolta, Minolta cm 3500 d. Co, Japan) and expressed as Hunter colour values (Hunter, 1975). Prior to measurement, the instrument was calibrated with white standard tile supplied by the manufacturer. Taking the average of five readings compensated surface pigmentation variation for each sample. Chroma, Total colour difference (TCD) and Hue angle were calculated from Hunter L, a, b values according to the formulae:

Chroma = $(a^2 + b^2)^{1/2}$

TCD = $[(Lo - L)^2 + (ao - a)^2 + (bo - b)^2]^{1/2}$

Hue angle = $\tan^{-1}$ (b/a), where subscript 'o' refer to the initial value

Chemical analyses

Total soluble solids (TSS) content of the fruits was determined using a hand refractometer (ERNA, Tokyo, Japan), the pH was measured with a pH meter using glass electrode (Beckman, USA), titratable acidity (as g/100g) of citric acid and vitamin C, as mg of ascorbic acid were determined by titrimetric method (Ranganna, 1991), reducing sugar by the DNS method (Miller, 1959) and was expressed as mg of reducing sugar as glucose per g of pulp. Total sugar was determined by the phenol-sulphuric acid method (Dubois *et al.*, 1956). Headspace gas CO_2/ O_2 was measured using PBI Dansensor CO_2, O_2 gas analyzer (Checkmate 9900, Denmark). Stastical analysis was carried out using standard methodologies with Microsoft Excel (Microsoft Corporation, USA, 2000).

Storage of *peda*

Freshly prepared branded milk based *peda*, weighing about 25-26 g was purchased from the local Diary outlets. They are packaged (5 pieces) in boxes under four conditions viz.1) in the usual card board carton (12 x 10 x 3 cm) used for *peda*, 2) in the usual carton in which a window (9.5 x 7.5 cm) was cut open in the lid, which was covered with butter paper (100 μ thick), 3) chitosan coated butter paper sheet, or 4) chitosan film. Cellophane tape of good quality (1.5 to 2 cm width) was used to secure the lid to the box, which was kept at room temperature (27 °C, 65% RH). Periodical observations on changes in characteristics of *peda* were determined. Colour was measured in Minolta colour measuring instrument and hue angle (θ) as $\tan^{-1}$(b/a) were calculated for comparing the changes in yellowness of the products. Texture (firmness) was measured using LLOYDS universal testing machine, by penetration mode using conical plunger to a depth of 10mm at a speed of 10

mm/min. Moisture loss was determined using toluene distillation method. Acidity percentage was determined by titration method, microbiological load was for standard plate count, coliforms, yeast and mould according to standard procedure (Speeks methods) and expressed as log cfu /g. Sensory profiling of the samples was carried out with the help of trained panel according to Quantitative Descriptive Analysis (QDA) method with a 15 cm scale anchored at 1.25 and 13.75 cm for detection and saturation threshold, respectively.

Wheat Flour

Commercial wheat flour procured from the local market was used for the studies. Moisture, total ash, dry gluten, falling number, sedimentation value, damaged starch, diastatic activity and farinograph characteristics of wheat flour were determined according to standard AACC methods (2000).

Preparation of bar cakes

The formulation and processing conditions for the preparation of bar cake are as follows.

Table 4.1 Formulations for preparation of bar cake

Ingredients	(g)
Wheat flour	100.00
Baking powder	0.50
Salt	0.50
Margarine	66.00
Sugar powder	100.00
Egg	120.00
Calcium propionate	0.50
Glacial acetic acid	0.20
Chitosan	0.0 / 0.1 / 0.2
Water	10
Cake gel	5.0

Processing conditions

Weighing the ingredients

Dissolving chitosan in acetic acid and calcium propionate in water and mixing together

Sifting of dry ingredients (flour, baking powder, salt)

Blending the above mixture with margarine till homogeneous (mixing 5 min)

Whipping egg, gel and sugar until the stiff foam stage (mixing time 7 min)

Adding egg in three parts to the flour margarine mixture (mixing 4 min)

Adding dissolved solution of chitosan, acetic acid and calcium propionate

Scaling the batter (450 g)

Baking (180^0C for 1 hr)

Cooling (2-3 hr)

Slicing

Evaluation of bar cakes

Bar cake weight was recorded; volume was determined using rapeseed displacement method (AACC, 2000). Sensory evaluation of bar cakes for crust colour, shape, crumb colour, grain and eating quality was carried out.

Storage studies of bar cakes

Bar cakes were prepared, cooled, sliced to half an inch thickness, packed separately using chitosan film, butter paper coated with chitosan and polypropylene pouches of 150-180 gauge and stored at room temperature. Bar cakes prepared with 0.1 and 0.2 % chitosan in the formulation were packed in polypropylene pouches of 150 – 180 gauge and stored at room temperature. Moisture in cakes was determined (AACC, 2000). Determination of crumb firmness of cakes according to AACC procedures (2000) using Texture Analyser (Model Tahdi, Stable Micro Systems, UK) under the following conditions: sample thickness: 25 mm, load cell: 10 kg, aluminium plunger diameter: 25 mm and plunger speed: 100

mm/min. Crumb firmness, which is a force at 25% compression was carried out at 1, 3, 5, 7, 9, 11, 13, 15 and 30 days of storage period. Cakes were observed for appearance of mold growth.

Preparation of plain and sweet breads

The following formulation was used for the preparation of plain and sweet breads

Table 4.2. Formulations for preparation of plain and sweet breads

Ingredients	Plain bread (g)	Sweet bread (g)
Flour	100	100
Yeast (compressed)	2	2
Salt	1.5	1.5
Sugar	3.0	20
Fat	3.0	2.0
Glycerol monostearate (GMS)	0.25	0.25
Sodium stearoyl-2-lactylate (SSL)	0.25	0.25
Calcium propionate	0.30 / 0.50	0.50
Acetic acid	0.10 / 0.20	0.20
Chitosan	0.0 / 0.10 / 0.20	0.0 / 0.20
Water	Variable	Variable

Plain and sweet breads were prepared according to the following conditions (Table 4.3)

Table 4.3. Processing steps for preparation of plain and sweet breads

Processing steps	Time (min)
Mixing (Hobart mixer)	4.0
Fermentation (86^0F, 75% RH)	90
Knock back	2.0
Fermentation	25
Sheeting and moulding	1.0
Proof (86^0F, 85% RH)	45 / 55
Baking (450^0F)	25

Evaluation of plain and sweet breads

Plain and sweet bread weight was recorded; volume was determined using rapeseed displacement method (AACC, 2000). Sensory evaluation of plain and sweet breads for crust colour, shape, crumb colour, grain, mouth feel and taste was carried out.

Storage characteristics of plain and sweet breads

Breads were cooled, packed separately using chitosan film, butter paper coated with chitosan and polypropylene pouches of 150 –180 gauge and stored at room temperature. Breads prepared with 0.0, 0.1 and 0.2 % chitosan in formulation were packed in polypropylene pouches of 150 – 180 gauge and stored at room temperature. Breads were stored till the appearance of mold growth. Moisture in breads was determined as per AACC procedure (2000). Determination of crumb firmness was carried out according to AACC procedures (2000) using Texture Analyser (Model Tahdi, Stable Micro Systems, UK) under the following conditions: sample thickness: 25 mm, load cell: 10 kg, aluminium plunger diameter: 25 mm and plunger speed: 100 mm/min. Crumb firmness which is a force at 25% compression was recorded.

Sensory analysis of fruits and vegetables

A Quantitative Descriptive Analysis (QDA) method of intensity scaling was used. QDA technique is an improvement over the categorical scaling, as it introduced greater objectivity to the measurement process, by using an internal scale of specified length anchored at both ends. The perceived attributes and words to describe them are derived by the panel and scores are converted to numbers by use of a template. In this technique of QDA, trained individuals identify and quantify in order of occurrence the sensory properties of a product or an ingredient. These data enable us to develop an

appropriate product multidimensional models in quantitative form that is readily understood in both marketing and R&D environment. These techniques have also been used successfully to develop concepts and idealize products before the actual developmental efforts are initiated (Stone *et al.*, 1974).

Trained panelists (12 members) who had experience in colour, texture and taste profiling of food products participated in the evaluation. The descriptors were derived after initial "Free choice profiling" where the panelists were asked to describe the samples with descriptive terms suitable for the samples. The common descriptors chosen by more than one third of the panelists were used for development of a `score card', which consisted of 15 cm scale in which 1.25 cm and 13.75 cm were arbitrarily anchored for `Recognition threshold' and `Saturation threshold', respectively for the attributes. Evaluation was carried out in a sensory laboratory with individual booths under fluorescent lighting similar to daylight. Three digit coded porcelain plates were used to serve the samples and panelists were asked to indicate the perceived intensities of the attributes by drawing a vertical line on the scale and writing the code number. Distilled water was used for rinsing and puffed rice for cleansing the palate between evaluations. The following attributes were selected for characterization of mango fruit. Colour of the fruit with respect to surface and cut surface, natural and defective spots; firmness and chewyness of the texture; and sweet, sour and astringency for taste were found adequate for describing the changes in tomatoes and bell pepper quality during storage.

Principal Component Analysis (PCA)

PCA is a very popular multivariate analytical technique that can be applied to QDA data to reduce the number of dependent variables (attributes) to a smaller number of underlying variable (called factors) based on correlation matrix among the original variables. The resulting data can be

applied to profiling specific product characteristics comparing and contrasting similar products based on attribute's importance. Essentially, it projects an n-dimensional space onto a two dimensional plot, analyses the correlation structure in the data set, and identifies the axis along which the maximum variation occurs. A second principal axis is then identified orthogonal to the first axis, corresponding to the second greatest amount of variations, and so on. The new axes are linear combinations of the original axis, and the coefficient, or loading measure the importance of the original variables in each principal component. PCA is rapidly becoming a routine statistical procedure for analyzing sensory profile data (Powers, 1988; Lawless and Heymann, 1998; Piggott and Sharman 1986), and extensive examples of its use have been given in the literature (Gonzalez Vinas *et al.,* 2001; Kallitharaka *et al.*, 2001; Dever *et al.*, 1996; Kim *et al.,* 1995).

The results obtained from physical tests and sensory evaluation of the mango fruits were subjected to PCA. This method of multivariate analysis was used for making multiple comparisons between the samples and packaging conditions, and to determine to what extent the variations observed in the results were accounted for by the parameters studied. Analysis of variance was carried out according Duncan's multiple ranges Test (Duncan, 1955). Analysis of experimental results was carried out using `Statistica'99 software.

Results and Discussion

A. Fruits: Mango

Mango fruits were packed under different conditions and stored at ambient condition (Fig.4.1). Data pertaining to physiological loss in weight of mangoes during storage under different conditions are shown in Fig. 4.2. PLW was less in MAP1 in comparison to control fruits because of reduction of transpiration loss and respiration rate in the former. PLW in MAP1 and control fruits was comparable up to 10 days, but on 11th day the control

Photo 1 **Photo 2**

Photo 3 **Photo 4**

Fig. 4.1. Mangoes stored for different periods (Photo 1 – Initial, Photo 2 – after 8 days, Photo 3 – after 12 days and Photo 4 – after 18 days)

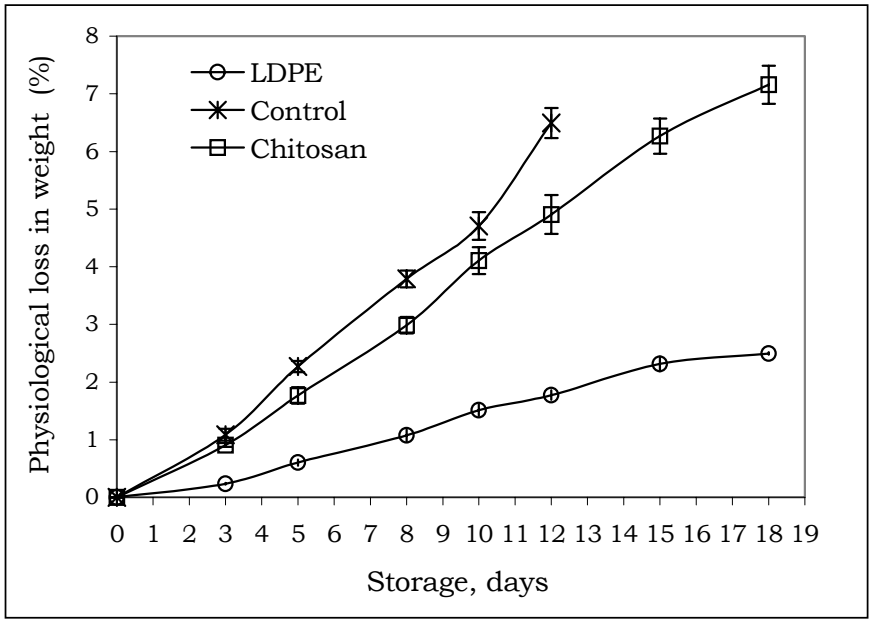

Fig. 4.2. Physiological loss in weight of control and packaged mango fruits during storage at 27 ± 1 °C

fruits showed small black patches due to microbial growth and on day 12, all the fruits were spoiled, whereas MAP1 fruits continued to remain greener and fresh till day 18 (fig.4.1). MAP2 fruits showed lesser weight loss (3.5%) compared to MAP1 (7.5%), probably due to reduction in transpiration of water vapour in LDPE films. The latter was observed as condensed water vapour droplets adhering to the inner surface of the LDPE films inside the boxes. The control fruits started spoiling from 11th day onwards, MAP2 fruits showed off-flavour and fungal growth after 12 days, but MAP1 fruits did not show any spoilage during the entire storage period of over 18 days (Fig.4.1), which also indicated the antimicrobial property of chitosan films in extending the shelflife of the fruit. It is also possible that this activity is related to the lower relative humidity in the chitosan-covered boxes, as seen with LDPE (fig.4.1). This is of importance in the transportation of produce to far-off places.

Accumulation of CO_2 and depletion of O_2 were determined as a percentage of total headspace to find out the effect of various holding conditions on the rate of respiration (Table 4.4). On day 3, the CO_2 and O_2 levels were 26.60% and 3.87% in MAP1 and 23.55% and 5.19% in MAP2, respectively. Then on the results showed a decreasing trend for CO_2 and an increasing trend for O_2 up to 10 days, and there after no significant.

Table 4.4. Changes in CO_2 and O_2 concentration in packaged mango fruits

Storage (days)	MAP 1		MAP 2	
	CO_2	O_2	CO_2	O_2
3	26.60 ± 0.00	3.87 ± 0.34	23.55 ± 0.92	5.19 ± 0.71
5	24.45 ± 0.21	3.94 ± 0.19	24.25 ± 0.00	5.72 ± 0.60
10	21.15 ± 0.07	5.60 ± 0.28	19.60 ± 0.71	6.28 ± 0.44
12	21.20 ± 0.14	5.53 ± 0.28	19.60 ± 0.71	6.28 ± 0.44
15	21.20 ± 0.14	4.90 ± 0.43	19.30 ± 0.57	5.96 ± 0.40
16	21.30 ± 0.00	5.01 ± 0.30	19.15 ± 0.49	6.24 ± 0.33
18	21.50 ± 0.00	5.21 ± 0.01	18.35 ± 0.07	6.65 ± 0.03

differences were observed. On day 18, the CO_2 and O_2 levels in MAP1 were 21.50% and 5.21%, and in MAP2 18.35% and 6.65%, respectively. A similar trend of CO_2 and O_2 has been observed in low temperature storage studies of mango (Gonzalez-Aguilar *et al.*, 1997; Dhalla and Hanson, 1998).

Textural data of the stored mango fruits are shown in Figs. 4.3-4.5.

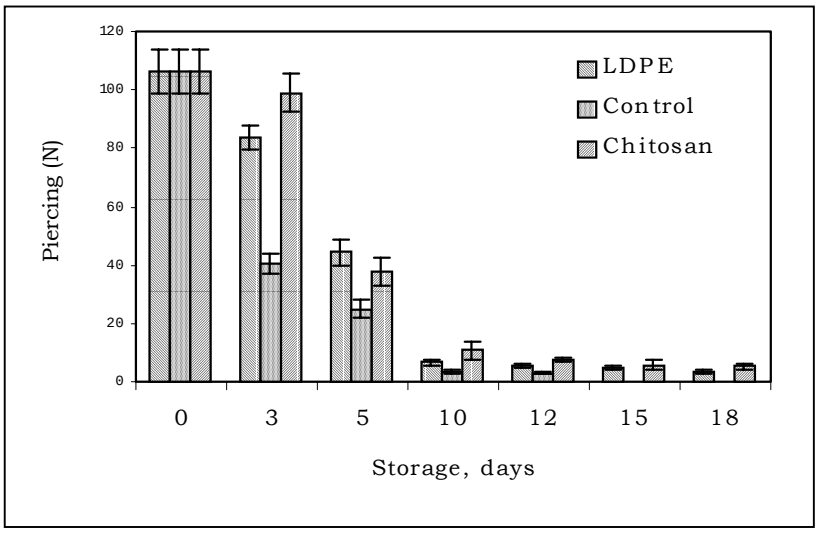

Fig. 4.3. Textural changes in piercing of control and packaged mango fruits during storage at 27 ± 1 °C

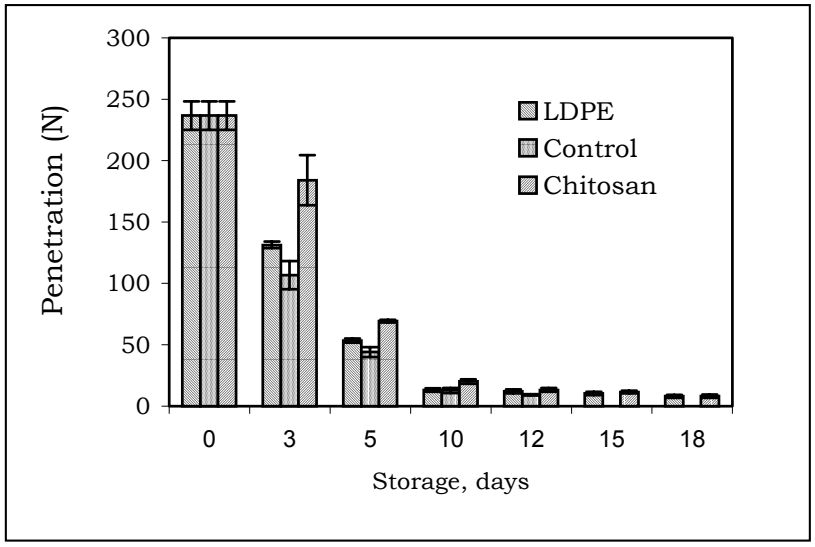

Fig. 4.4. Textural changes in penetration of control and packaged mango fruits during storage at 27 ± 1 °C

The piercing value showed a loss of 60% on third day of storage, compared to 10% loss in MAP. On 10th day control fruits ripened and force is found to be less than 10 N (Fig. 4.3). Similar trend was observed in penetration mode also (Fig.4.4). A 50% loss of compression force was seen in control fruits on day 3 (Fig.4.5). A similar trend, but at a lower rate was observed in packaged fruits. During the entire storage period the MAP fruits showed better rupture force than control fruits.

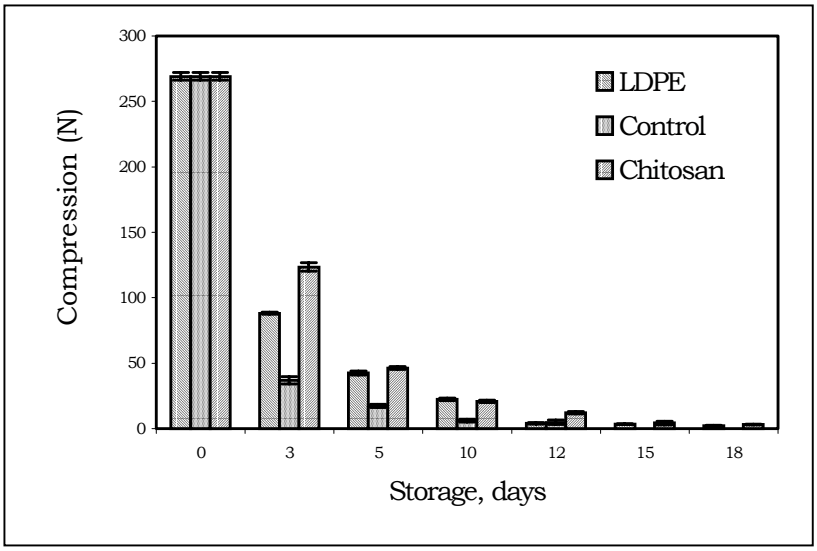

Fig. 4.5. Textural changes in compression of control and packaged mango fruits during storage at 27 ± 1 °C

The relatively higher 'a' negative values (see Table 4.5) were indicating the greenness of fruits sealed with LDPE or chitosan films even after 10 days, which decreased rapidly during further storage, but the 'a' values were positive for the fruits packaged in the perforated plastic box indicating redness of colour by 10 days of storage and it showed little change after 12 and 15 days. The 'b' values representing the yellowness of the fruit, was lowest immediately after harvest and increased for fruits under any condition during storage. The values were slightly lower for the fruits sealed with chitosan films up to 12 days and were comparable to that recorded for other

Table 4.5. Change in colour of control and packaged mango fruits

Days	L	a	b	Chroma	TCD	Hue Angle
CONTROL						
0	40.02	-11.96	16.31	20.22		-36.25
3	50.68	-12.50	25.07	28.01	13.81	-26.50
5	57.99	4.40	32.09	32.39	28.98	7.80
10	57.25	12.47	31.18	33.58	33.39	21.79
12	56.57	13.99	32.04	34.96	34.57	23.59
MAP1						
0	40.02	-11.96	16.31	20.22		-36.25
3	40.53	-11.74	16.93	20.60	0.83	-34.73
5	43.41	-11.81	18.79	22.19	4.20	-32.15
10	40.45	-11.25	16.93	20.32	1.04	-33.60
12	46.50	-7.83	16.93	18.65	7.71	-24.82
16	51.93	-7.09	26.22	27.16	16.24	-15.13
18	52.31	-3.09	27.52	27.69	18.85	-6.40
MAP2						
0	40.02	-11.96	16.31	20.22		-36.25
3	47.85	-13.09	23.22	26.66	10.52	-29.41
5	45.46	-12.45	20.88	24.31	7.12	-30.80
10	48.52	-10.48	25.46	27.54	12.58	-22.37
12	51.49	-4.53	29.81	30.15	19.21	-8.78
16	54.62	-2.98	30.26	30.41	22.10	-5.63
18	52.05	0.94	30.77	30.78	22.81	-1.75

fruits after 15 days of storage. The 'L' value representing the lightness of colour was lowest in the beginning, and this along with the negative value for 'a' indicated the intense green colour of the fruits soon after harvest. The 'L' value was highest for fruits packaged in plastic box followed by those sealed with LDPE films and lowest for those sealed with chitosan films after 10 days of storage. The changes were marginal after 12 days but increased after further storage. The relative changes in 'L', 'a' and 'b' values indicated that ripening was delayed in the fruits packaged with chitosan films (Table 4.5).

No significant changes in colour were observed in MAP1 and MAP2 stored fruits (Table 4.5). After 5 days, the hue angle value of the control fruits shifted to first quadrant concomitant with the change in colour of the fruit from green to yellow. On day 8 there was a uniform (yellow) colouring in control fruits, whereas the packaged fruits showed better retention of green

colour throughout storage. The hue angle values of packaged fruits were distributed in second quadrant up to 18 days indicating that the fruits maintained green colour. Retardation of chlorophyll degradation in such fruits may be due to high CO_2 and low O_2 levels in the headspace. These results were comparable with the earlier report (Kolekaret *et al.*, 1992), wherein a delay in the colouring of mango fruits coated with sucrose esters was noticed. The retention of chlorophyll by MAP1 packaging indicates the effect of chitosan films in retarding the ripening process and thus allowing extension of shelflife.

The TSS increased with the storage period, but throughout MAP1 packed fruits showed a lower value compared to MAP2 and control fruits (Fig. 4.6). Lower TSS value (means a lower ripening rate) in MAP1 is probably

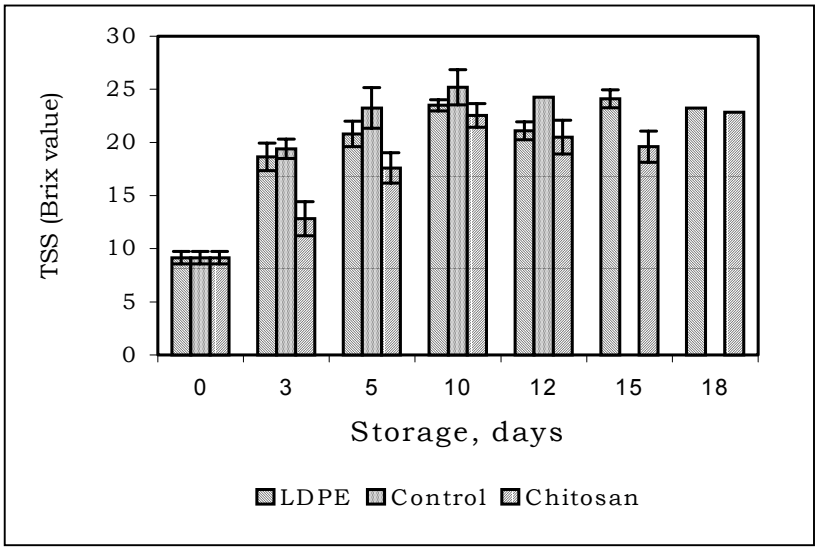

Fig.4.6. Changes in total soluble solids (TSS) of control and packaged mango fruits during storage at 27 ± 1 °C

due to its higher WVTR as compared with MAP2. The formation of TSS takes place due to the break down of complex carbohydrates into water-soluble sugars. MAP1 fruits showed little decrease on 12th day and maximum on 15th day, contrary to a maximum TSS value on day 10 for control fruits and day 15 for MAP2 fruits.

Initially the pH of the fruits was 4.06, but as the fruits ripened, the pH of control fruits increased to 6.73 on day 12 compared to MAP1 (5.04) and MAP2 (5.79) fruits (Fig. 4.7). The titrable acidity values (Fig. 4.8) also showed a decreasing trend with the initial value of 2.17, which got reduced to 0.08 in control fruits on day 12. In the case of MAP fruits this reduction was much lower as reported earlier (Gonzalez-Aguilar *et al.*, 1997; Dhalla and Hanson, 1998).

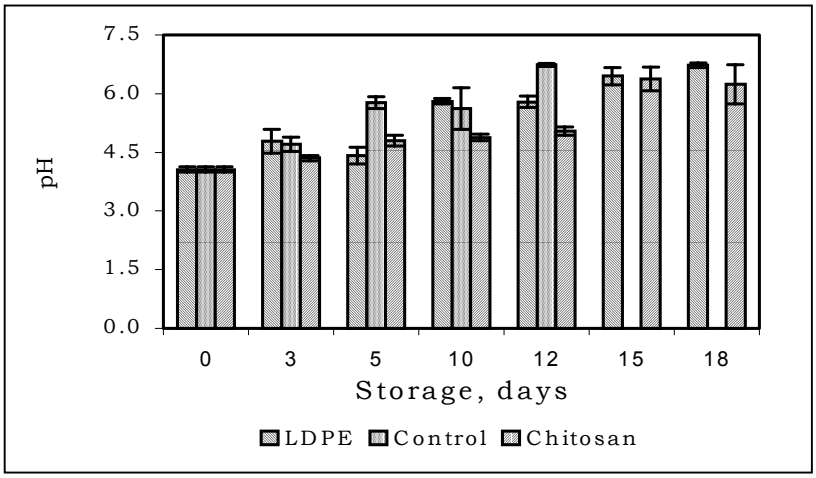

Fig. 4.7. Changes in pH of control and packaged mango fruits during storage at 27 ± 1 °C

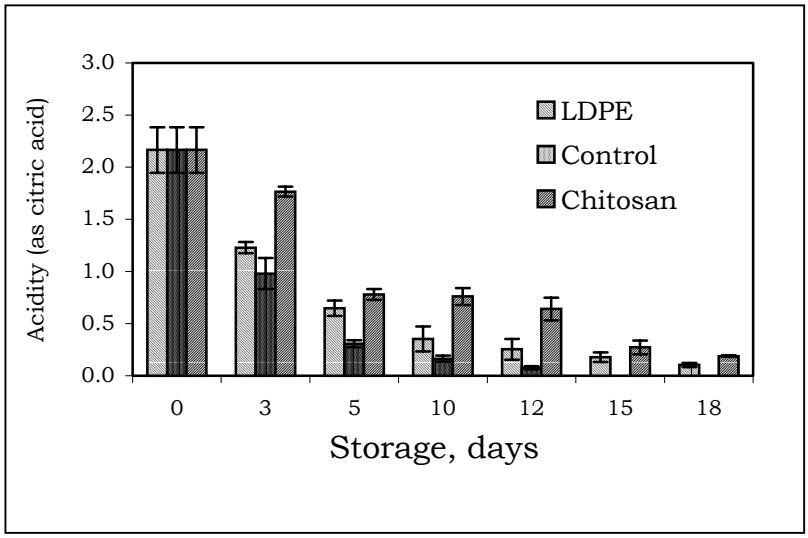

Fig. 4.8. Changes in titrable acidity of control and packaged mango fruits during storage at 27±1 °C

The change in pH is attributed to the formation of sugar, acids etc. during ripening. The moisture content of the pulp was 70% at the harvest period and it increased to 80% on day 6 followed by a slight reduction (Fig. 4.8). No significant difference was observed in control fruits when compared with packaged fruits

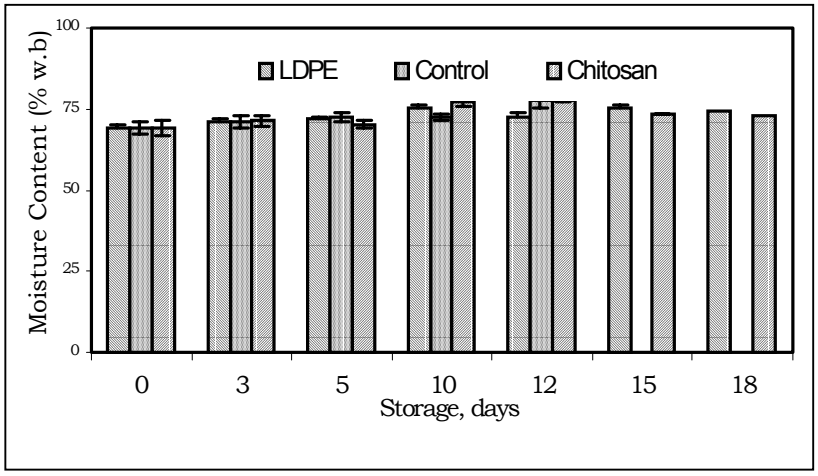

Fig. 4.9. Changes in moisture content (%) of control and packaged mango fruits during storage at 27 ± 1 °C

Throughout the storage period the total sugar (TS) level was more than the reducing sugar (RS) (Figs. 4.10 and 4.11). Both of them showed an

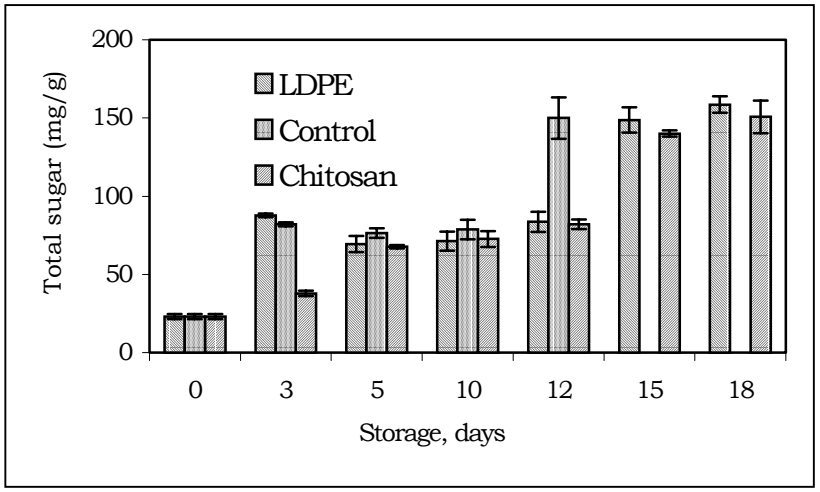

Fig. 4. 10. Changes in total sugar (TS) of control and packaged mango fruits during storage at 27 ± 1 °C

increasing trend. The initial levels of TS and RS were 23.00 and 16.17 mg/g of pulp, respectively. Control fruits gave a maximum sugar level on day 12 (150 mg/ g of pulp) whereas MAP2 packaged fruits gave maximum values on day 16, but no significant difference in TS was observed in MAP1 fruits, as reported before (Miller *et al.*, 1983).

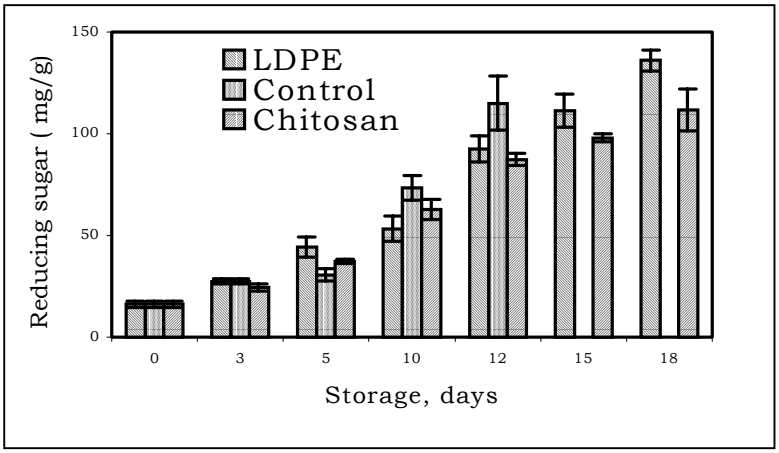

Fig. 4.11. Changes in reducing sugar (RS) of control and packaged mango fruits during storage at 27 ± 1 °C

Initially fruits had a higher concentration of vitamin C (115 mg/100 g of pulp), which decreased during storage (Fig.4.12). Vitamin C retention was more in control fruits than in packaged fruits, which may be due to the higher concentration of CO_2 inside the package.

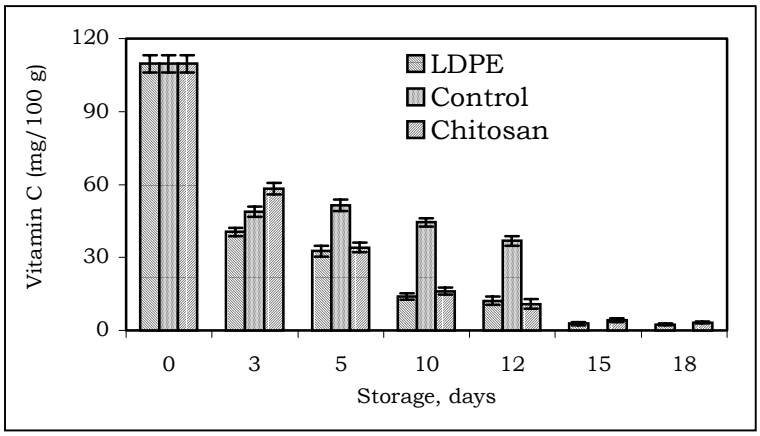

Fig. 4.12. Changes in vitamin C of control and packaged mango fruits during storage at 27 ± 1 °C

Chlorophyll level at different days of storage is shown in Fig. 4.13 The control fruits turned yellow on day 8 and appeared golden yellow on day 10. MAP fruits showed a gradual decrease in chlorophyll level. Carotenoid value

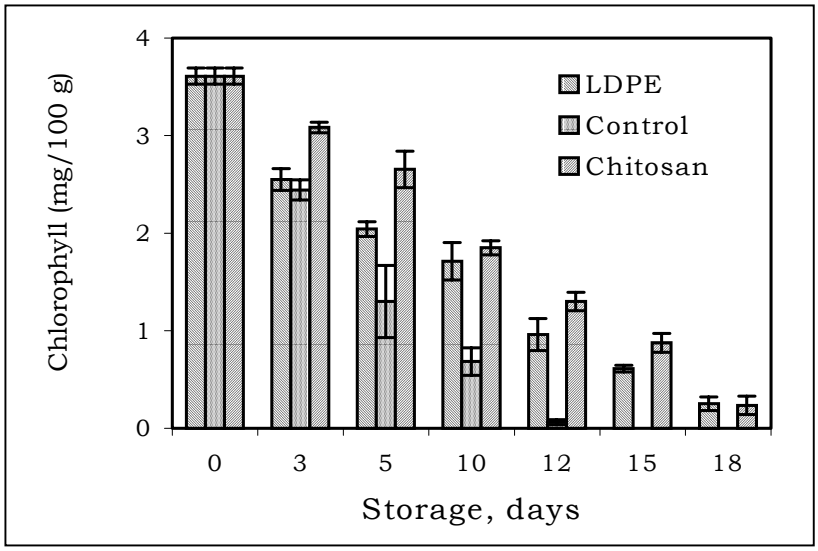

Fig. 4.13. Changes in chlorophyll content of control and packaged mango fruits during storage at 27 ± 1 °C

of raw fruits was 1696.61 µg/ 100 g of pulp, and increased by 9 folds during the storage period (Fig. 4.14) indicating a progressive ripening of the fruits.

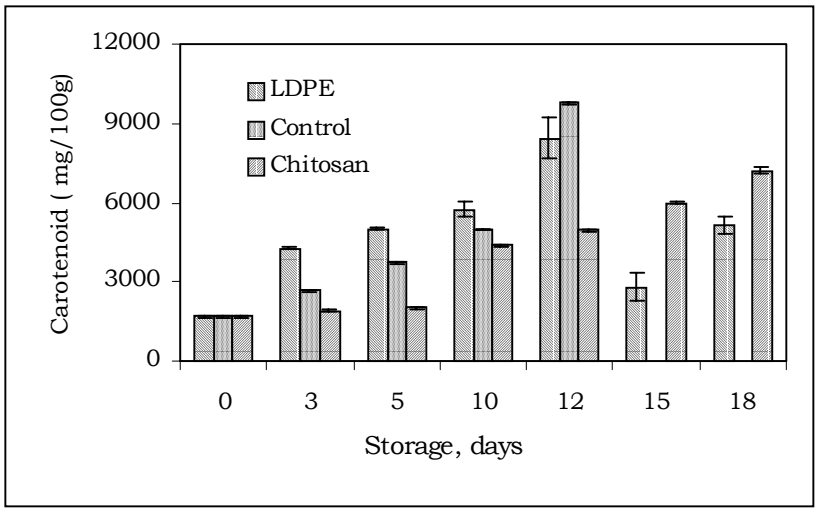

Fig. 4.14. Changes in carotenoid of control and packaged mango fruits during storage at 27 ± 1 °C

In all fruits the carotenoids content showed an increasing trend. It was more in control fruits compared to MAP2 fruits, which showed a higher value on 12th day and later decreased. In MAP1, carotenoid content showed a maximum value of 7280.40 µg/ 100 g of pulp on day 18. Gradual decrease in stored fruits may be due to higher level of CO_2.

As the fruits reached the ripening stage, they were screened for any off-flavour development, CO_2 injury and other physiological changes. MAP2 fruits showed more off-flavour. It is reported that fruits packed with LDPE (without perforation) develop off-flavour (Alves *et al.*, 1998). Similarly raw mango sealed in airtight polyethylene bags when removed from the bag and allowed to ripen developed strong off-flavour (Grantly *et al.*, 1982). Though initially fungicidal treatment was given to all the fruits, the effect did not sustain for long storage period and they were spoiled. Control fruits decayed in 12 days, whereas packaged fruits showed increased shelflife. MAP1 fruits showed very little spoilage. Condensation of water inside the pack resulted in higher humidity leading to fungal growth in fruits packed with LDPE.

Sensory evaluation of mango fruits

Fruits stored for 10 days in perforated plastic box were not moist on the surface and showed some natural spots with randomly distributed zones of orange and greenish orange colour. After 10 days, the raw, sour and astringent odor decreased with the development of orange colour and typical mango flavor. In the LDPE film sealed cartons condensation of water droplets on the inner film surface was noticed and fruits were also moist on the surface, but this type of condensation was absent in the cartons sealed with chitosan films.

The mean scores for various sensory attributes of the fruits during storage are shown in Table 4.6. Fruits stored in perforated plastic box showed the development of surface colour, cut surface colour and sharpness, mango flavor and sweet notes by 10 days of storage, and the raw, sour and

astringent notes decreased. The fruits were good for consumption up to 12 days of storage, but by 15 days they became soft with more defective spots as compared to 12 days of storage. Although sweetness was not affected, it was slightly chewy (Table 4.6). Fruits stored in LDPE film sealed cartons were still greenish in colour with sour, raw and astringent notes being perceived even after 12 days of storage. The sweet and ripe mango aroma notes appeared between 12–15 days of storage, as compared to initial or 10 days of storage. The fruits had desirable sweetness and cut surface colour, but slightly chewy. Beyond 15 days, the fruits became too soft and defective spots covered the fruit surface to a greater extent rendering them unfit for consumption (Table 4.6). The fruits stored in chitosan film sealed cartons showed development of mango aroma with good sharpness of cut surface of fruit by 10 days (data not shown), but the fruit colour and cut surface colour with desirable sweetness and typical mango flavor were more by 12 days of storage, and values were very similar for fruits stored up to 15 days (Table 4.6). The desirable colour, firmness and taste were retained up to 18 days of days of storage (data not shown), but by 20 days the desirable notes showed a slight decrease. Beyond 21 days although some of the desirable qualities were retained, the surface was covered with some natural spots and very few defective spots were observed, they became slightly chewy although the sweetness was high between 21 to 22 days of storage (Table 4.6).

The development of desirable colour or cut surface colour, sweetness and mango flavor are the major attributes contributing to fitness of fruits for consumption, while development of defective spots and chewy notes were the attributes rendering them less fit for consumption. Reduction in sour, raw and astringent notes showed greater changes in the fruits stored under different conditions. Sensory scores revealed that chitosan film can extend the storage life of fruit to a longer period.

Table 4.6. Mean scores of sensory attributes for mango fruits during storage

Packaging material	Storage Days	Colour	Spot Natural	Spot Defect	Cut colour	Sharp	Raw	Mango	Firm	Chewy	Sweet	Sour	Astringent
Initial	0	2.70^{a}	5.95^{a}	1.5^{a}	4.91^{a}	7.93a	7.66^{a}	5.14^{a}	8.66^{a}	1.60^{a}	5.03^{a}	6.23^{a}	3.95^{a}
LDPE film	10	5.40^{b}	6.13^{a}	3.44^{ab}	6.17^{ab}	7.29^{b}	4.36^{b}	6.82^{b}	7.88^{ab}	3.3^{b}	7.18^{b}	3.94^{b}	1.46^{b}
	12	7.45^{c}	6.38^{a}	8.85^{c}	8.05^{bc}	6.75^{c}	3.81^{bc}	7.51^{bc}	6.38^{c}	6.38^{d}	8.25^{c}	1.48^{c}	1.14^{bc}
	15	8.14^{cd}	7.77^{a}	8.56^{c}	8.17^{bc}	6.41^{c}	3.55^{bc}	7.89^{bc}	6.19^{c}	6.50^{d}	8.39^{c}	1.50^{c}	1.08^{bc}
Chitosan film	10	6.92^{bc}	6.25^{a}	2.31^{a}	7.5^{b}	7.60^{b}	3.98^{b}	8.17^{c}	8.32^{a}	3.92^{bc}	7.33^{b}	2.25^{bc}	0.96^{c}
	12	7.12^{bc}	6.55^{a}	2.31^{a}	7.95^{bc}	7.35^{b}	3.67^{bc}	8.36^{c}	7.69^{ab}	4.85^{c}	7.56^{bc}	1.85^{c}	0.93^{c}
	15	7.48^{c}	7.01^{a}	2.35^{a}	8.45^{bc}	7.33^{b}	3.67^{bc}	8.42^{c}	7.59^{ab}	5.45^{cd}	8.22^{c}	1.78^{c}	0.93^{c}
	20	7.95^{c}	6.85^{a}	3.88^{b}	8.54^{bc}	6.71^{c}	2.89^{c}	8.59^{c}	7.16^{b}	5.55^{cd}	8.41^{c}	1.68^{c}	0.91^{cd}
Plastic box	10	9.86^{d}	8.22^{a}	6.83^{c}	9.5^{c}	7.62^{b}	3.24^{c}	8.41^{c}	7.54^{ab}	4.62a^{cd}	7.5^{b}	1.98^{c}	0.88^{d}
	12	10.07^{d}	8.3^{a}	6.94^{c}	9.75^{c}	7.72^{b}	2.86^{c}	8.51^{c}	7.31^{ab}	4.72a^{cd}	8.07^{bc}	1.71^{c}	0.85^{d}
	15	10.5^{d}	8.5^{a}	7.5^{d}	10.02^{c}	6.73^{c}	2.86^{c}	8.31^{c}	6.58^{c}	4.92a^{cd}	8.17^{bc}	1.70^{c}	0.83^{d}

Mean values in a same column with different superscripts differ significantly ($p<0.05$)

PCA for sensory attributes

The PCA plot of the data is shown in Fig.4.15. The axis PC 1 accounts for 74.48% of the variance and PC 2 for 13.05% of variance, and together they account for 87.53% of the total variance observed. The desirable notes were mostly negatively loaded, while attributes like raw, sour and astringent notes were positively loaded. In PC 2 most of the desirable notes had positive loading, while chewy, raw, development of defective spots and astringent notes were negatively loaded. However PC 3 (in figure not shown) accounted for 8.5% of the observed variance.

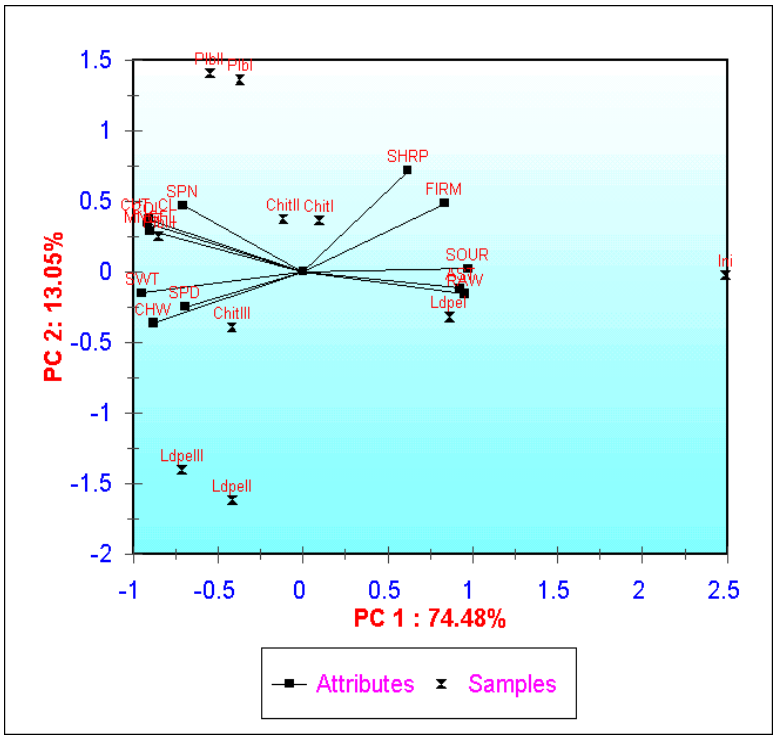

Fig. 4.15. PCA plots of mango sensory attributes during storage

It is seen that the fruits stored under different conditions occupy different quadrants indicating that they differ in their quality attributes. Fruits stored in the plastic box and chitosan film sealed cartons were close to desirable quality attributes, while LDPE covered fruits had still some greenish yellow surface appearance and other attributes such as sour, raw

and astringent notes related to slightly unripe fruits. After 15 days of storage, fruits stored in plastic baskets were slightly chewy, although had desirable colour and mango aroma and sweetness, they were less satisfactory for consumption, as they had developed more defective spots. The LDPE and chitosan film covered fruits also differed considerably in that the latter were positioned close to desirable quality attributes with no defective spots or other undesirable changes, while the former showed lower raw, astringent and sour notes, but had developed some defective spots with slight chewy note. During storage beyond 18 days for fruits stored with LDPE film and beyond 21 days for fruits stored with chitosan film, the fruits became less suitable for evaluation. Fruits stored with LDPE films became slightly chewy and developed more defective spots although they had comparable colour, mango aroma and sweet notes, while chitosan film covered fruits have retained most of the desirable quality attributes though some of them developed defective spots. Thus, the desirable quality of some of the fruits covered with chitosan film was still good even after 21 to 22 days (data not shown).

PCA for physical and sensory attributes

Data related to changes in both physical and sensory properties are shown as PCA plot in Fig.4.16. The axis PC 1 accounts for 65.02% of the variance and PC 2 for 20.15% of variance, and together they account for 85.17% of the total observed variance. Among the physical properties, only CO2 and O2 % levels showed lower negative values, and others had higher negative values in PC1 and only firmness had a positive value in both PC1 and PC2. All the other factors had higher positive value in PC2. Among the sensory attributes most of the undesirable notes had positive value in PC1 and lower negative value in PC2. The sourness and pH, sourness and sweetness, firmness (instrumental) or by sensory evaluation and chewy notes were oppositely loaded in PC1 and PC2, indicating good correlation between

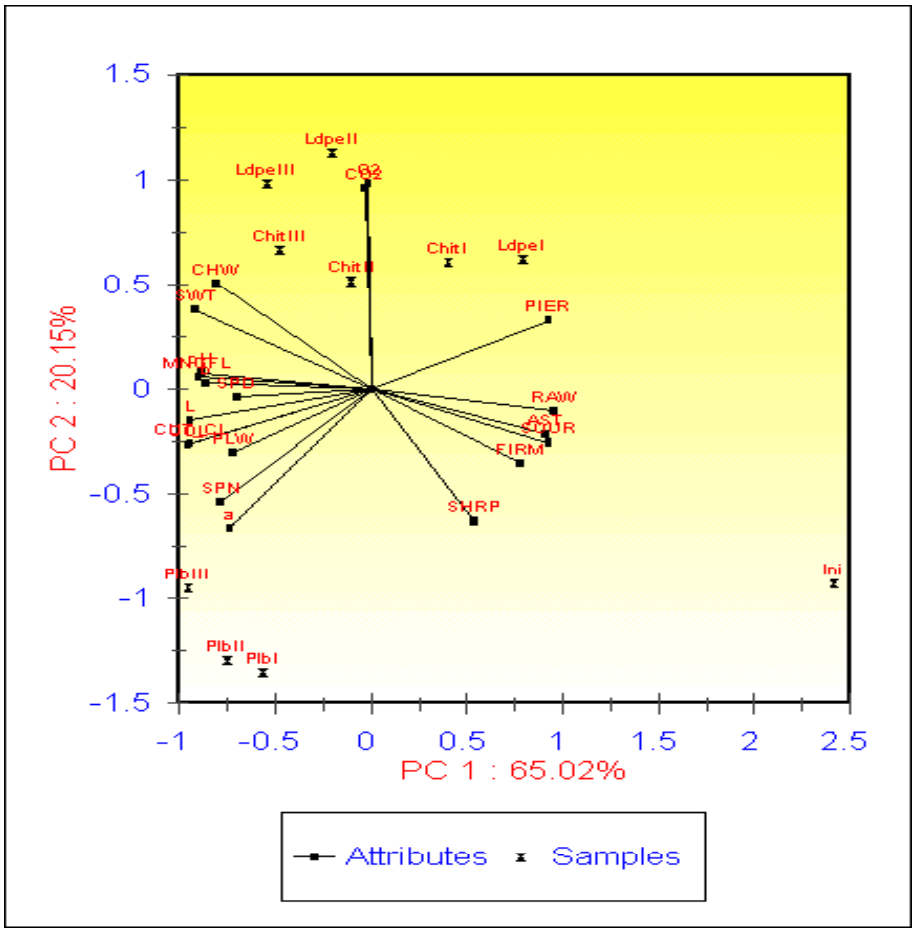

Fig. 4.16. PCA plots of physical and sensory attributes of mango during storage

attributes. The loadings also indicate the differential segregation of desirable and undesirable attributes.

It is seen that in PC1, force required for indicating the firmness associated with the fruits, raw, sour and astringent notes were having positive values, and the values for fruits after harvest were closer to these attributes. The location of the fruits at initial stage of storage closely matched this. The appearance of natural spots, weight loss during storage, colour, mango aroma, and sweetness had negative values. Fruits stored under any condition followed this pattern during ripening. The relative

difference exhibited by the fruits during storage is indicated by the differences in PC2. The fruits packaged in perforated plastic box which is in equilibrium with the atmospheric gas pressure had negative values and differed from those exposed to modified atmosphere which had positive values. The sweetness and chewy notes that increase with ripening are associated with the fruits stored for longer duration. The fruits stored with LDPE film had higher values for chewy notes and lower values for firmness. Firmness and chewy notes, sourness and pH showed a negative correlation. The colour of whole fruit or cut surface as measured either by sensory or instrumental method was similar and closely located. The distribution of sample location with respect to the attributes clearly highlight the advantage of chitosan based film during storage in extending the shelflife of mango fruits, as they retained the desirable quality attributes for a relatively longer period. The fruits stored in perforated plastic box showed about 30-40% decay after 12 days of storage as the loss of firmness and formation of defective spots were observed. This type of loss is about 35-45% with respect to fruits stored with LDPE films after 15 days of storage. However, this type of decay was very low in case of fruits stored with chitosan film as the fruits were free from these symptoms even upto 15–18 days of storage and showed only 10-12 % of the decaying symptoms after 20-22 days of storage.

Conclusions

The results of this study clearly indicate that mango fruits stored in wax lined cartons sealed with chitosan films have a longer shelflife and retain desirable quality attributes at a higher level as compared to fruits stored in wax lined cartons sealed with LDPE films or in perforated plastic box. The higher level of carbon dioxide and lower level of oxygen or lower rate of oxygen transmission associated with chitosan films delays ripening and higher water vapor transmission rate minimizes the rate of transpiration and prevents condensation of water droplets on the film and thus helps in

establishing equilibrium moisture content of mangoes in the cartons which in turn extend the shelflife of fruits for a longer period.

B. Vegetables: Tomatoes and Bell pepper

The effect of modified packaging of tomato and bell pepper is shown in Fig.4.17. Tomatoes packed with chitosan film (Fig.4.17. [5]) showed uniform colour development compared to LDPE packed fruits (Fig. 4.17. [4]). Whereas LDPE stored in bell pepper showed better colour development (Fig.4.17 [3]) compared to chitosan packed pods (Fig.4.17. [1,2]). Effect of modified atmosphere package on O_2 and CO_2 levels of tomato and bell pepper during storage is shown in Table 4.7. In general the level of O_2 showed marginal.

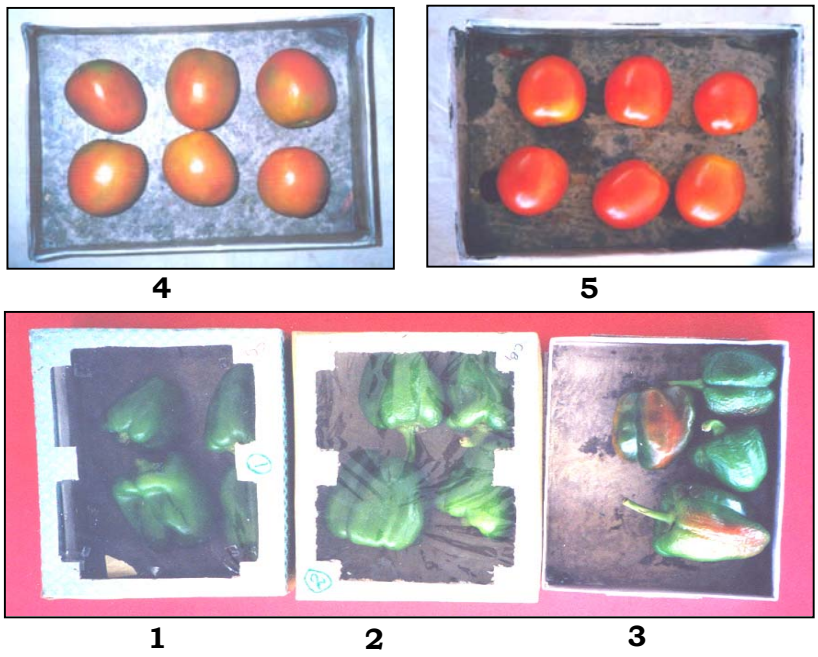

Fig. 4. 17. Tomato and bell pepper stored for different periods (Photo 4 – LDPE, Photo 5 – after 30 days (chitosan film), after 16 days of storage bell pepper, chitosan flm (1-2) and LDPE (3)

Table 4.7. Changes in CO_2 and O_2 concentration in packaged tomato and bell pepper

	Days	Chitosan		LDPE	
Tomatoes		CO_2	O_2	CO_2	O_2
	4	1.0 ± 0.60	21.8 ± 0.4	1.13 ± 0.50	20.3 ± 0.6
	8	1.6 ± 0.42	20.34 ± 0.6	2.21 ± 0.46	20.2 ± 0.45
	11	2.1 ± 0.60	20.4 ± 0.8	2.60 ± 0.60	21.7 ± 1.2
	16	2.2 ± 0.40	21.1 ± 0.5	2.16 ± 0.4	21.2 ± 0.86
	21	2.2 ± 0.2	20.8 ± 0.6	2.2 ± 0.2	20.7 ± 0.8
	28	2.6 ± 0.20	20.9 ±. 2	2.2 ± 0.4	20.4 ± 0.6
Bell pepper					
	5	1.24 ± 0.64	20.6 ± 0.68	1.0 ± 0.64	20.8 ± 0.62
	10	1.2 ± 0.46	21.2 ± 0.82	1.0 ± 0.24	20.4 ± 0.84
	15	1.5 ± 0.46	20.9 ± 0.62	2.2 ± 0.8	21.7 ± 0.46
	16	1.6 ± 0.24	20.7 ± 0.64	2.2 ± 0.6	22.1 ± 0.78

Changes, while that of CO_2 showed a gradual increase. The values were ranged from 1 to 2.6 % for tomato and 1.2-1.6% for bell pepper in chitosan film covered cartons and 1.13 to 2.2% for tomato and 1-2.2% for bell pepper in LDPE covered cartons. High CO_2 levels delayed loss of green colour (Exama *et al*, 1993; Otma, 1989) and also resulted in calyx discolouration (Khudairi, 1972). In the present study the level of CO_2 did not cross 3% under any condition during the storage period.

Weight loss during storage differs according to the nature and constituents of vegetables. Weight loss was lower under packaged conditions compared to unpackaged fruits (Fig. 4.18 a & b). In tomatoes 17-18% loss in unpackaged, 13-14 % loss in chitosan film covered fruits and 4-5% loss in LDPE covered fruits upto 21 days of storage were observed. By 30 days, unpackaged fruits were spoiled while chitosan and LDPE film covered fruits showed 17%-18% and 7-7.5% loss in weight, respectively. In bell pepper a similar trend was observed. The weight loss was 14-15% for unpackaged pods, 10-11% for chitosan film covered pod, and 2-2.5% for LDPE film covered pods upto 16 days of storage.

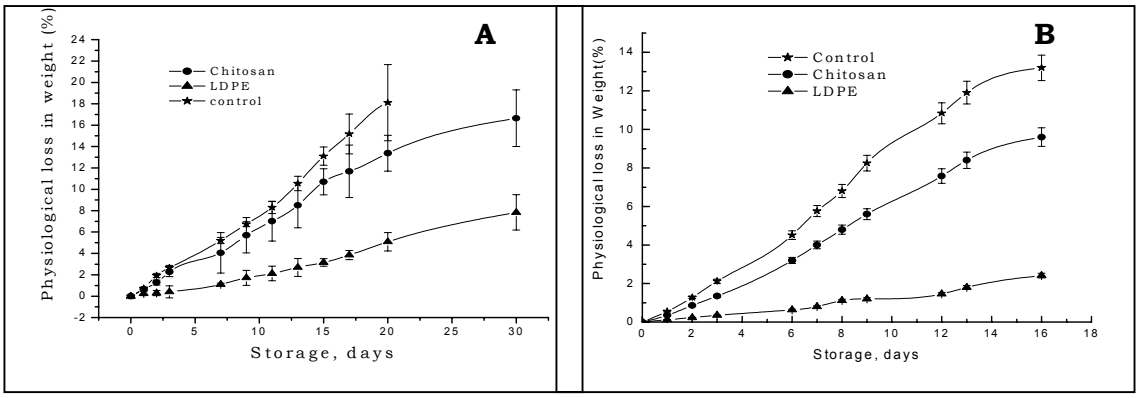

Fig. 4.18. Physiological loss in weight (%) of control and packaged tomato (A) and bell pepper (B) during storage at 27 ± 1 °C

Colour of tomatoes and bell pepper during storage

The colour changes during storage of tomato are shown in Table 4.8. It is associated with loss of chlorophyll and rapid accumulation of carotenoids,

Table 4.8. Colour values of tomatoes during storage

Days	L	a	b	RV	Hue angle (Degrees)
	Control				
0	45.11 ± 1.39	7.78 ± 2.45	22.38 ± 1.84	10.53 ± 2.13	72.18 ± 1.06
7	30.32 ± 12.26	21.07 ± 2.86	18.9 ± 3.14	28.27 ± 11.23	41.97 ± 3.06
14	32.79 ± 0.9	25.52 ± 2.61	14.19 ± 0.69	31.57 ± 2.33	28.94 ± 1.28
21	34.17 ± 2.72	22.81 ± 8.62	12.56 ± 5.06	34.32 ± 4.84	28.18 ± 0.78
	Chitosan				
0	45.11 ± 1.39	7.78 ± 2.45	22.38 ± 1.84	10.53 ± 2.13	72.18 ± 1.06
7	35.57 ± 0.16	24.92 ± 4.15	17.21 ± 9.82	31.75 ± 6.47	37.36 ± 1.24
14	34.32 ± 0.69	25.83 ± 1.49	15.42 ± 1.10	32.20 ± 2.95	34.24 ± 1.96
21	33.02 ± 0.77	24.83 ± 0.76	14.96 ± 0.76	32.76 ± 1.99	31.12 ± 0.76
30	36.28 ± 1.74	22.28 ± 2.87	16.52 ± 2.50	37.73 ± 3.53	29.54 ± 0.56
	LDPE				
0	45.11 ± 1.39	7.78 ± 2.45	22.38 ± 1.84	10.53 ± 2.13	72.18 ± 1.06
7	36.77 ± 3.27	23.54 ± 9.34	18.21 ± 9.21	31.78 ± 7.11	40.62 ± 2.04
14	36.49 ± 1.97	26.33 ± 1.91	15.00 ± 0.74	32.24 ± 7.02	34.66 ± 1.63
21	33.68 ± 1.12	23.28 ± 2.93	18.03 ± 2.69	32.42 ± 1.42	34.06 ± 2.48
30	38.67 ± 2.69	22.27 ± 1.76	19.13 ± 2.18	39.54 ± 5.34	29.72 ± 0.56

particularly lycopene, as chloroplasts are converted to chromoplasts and the greenish yellow colour is changed to red colour (Khudairi, 1972). The redness values increased from 10.53 to 34.32 and hue angle decrease from 72˚ to 28˚ degrees during storage period in unpackaged tomatoes and highest was observed at the end of 21 days of storage period. Packaged tomatoes were observed to develop higher redness value compared to unpackaged tomatoes by 7th day of storage, and this trend continued until 30 days of storage. In packaged tomatoes the greenish yellow colour changed to red colour as indicated by hue angle, which decreased to 29˚ from 72˚. The redness value increased from 10.52-39.9. Colour development of tomato was influenced by gas composition of its environment (Yang and Chinnan 1987), high CO_2 levels decreasing ethylene synthesis, which can delay colour changes (Buescher, 1979) in agreement with our observations.

The colour changes during storage of bell pepper (Table 4.9) did not vary much. The L value did not change much, 'a' value showed slight

Table 4.9. Colour values of bell pepper during storage

	Days	L	a	b	Hue angle (degrees)
Control	0	24.03 ± 2.54	-9.94 ± 1.13	14 ± 2.51	125 ± 1.65
	4	33.385 ± 3.37	-9.69 ± 1.43	12.43 ± 2.67	119.14 ± 4.56
	8	34.03 ± 3.43	-6.42 ± 1.13	14.10 ± 2.51	115.23 ± 2.96
	11	34.17 ± 2.72	1.07 ± 1.86	18.9 ± 3.14	86.75 ± 2.48
LDPE	0	24.03 ± 2.54	-9.94 ± 1.13	14 ± 2.51	125.00 ± 1.65
	4	32.93 ± 1.79	-9.45 ± 0.90	12.64 ± 1.51	126.78 ± 2.56
	8	36.42 ± 2.46	-10.97 ± 0.86	15.48 ± 2.08	125.32 ± 2.48
	11	33.75 ± 2.44	-8.92 ± 0.81	12.98 ± 2.10	124.49 ± 1.56
	16	37.31 ± 4.26	0.86 ± 1.8	16.83 ± 3.58	106.23 ± 2.04
Chitosan					
	0	24.03 ± 2.54	-9.94 ± 1.13	14 ± 2.51	125.0 ± 1.65
	4	35.05 ± 1.84	-10.29 ± 0.96	14.46 ± 1.69	125.43 ± 1.48
	8	32.11 ± 2.5	-6.74 ± 2.71	11.45 ± 2.13	120.38 ± 1.48
	11	32.59 ± 1.59	-8.84 ± 0.78	12.0 ± 0.97	126.37 ± 2.04
	16	32.54 ± 1.17	-8.84 ± 0.78	11.36 ± 1.48	113.48 ± 2.64

decrease, while ' b' value showed a marginal increase. The Hue angle decreased during storage and it showed a lowest value for unpackaged pods. Hue angle was close to the range of 100˚-120˚ until 8-10 days of storage, and then showed a greater decrease by 16 days of storage. Chitosan film covered pods retained green colour to a greater extent compared to LDPE film covered pods.

Textural analysis

Changes in texture of unpackaged and packaged tomatoes during storage period are shown in Fig.4.19. MAP significantly slowed the softening of tomato during the storage period. The penetration of tomatoes decreased during storage, the values were 17.24 N in unpackaged, 22.3 N in chitosan packaged and 21.8 N in LDPE packaged fruits. On 21st day the unpackaged

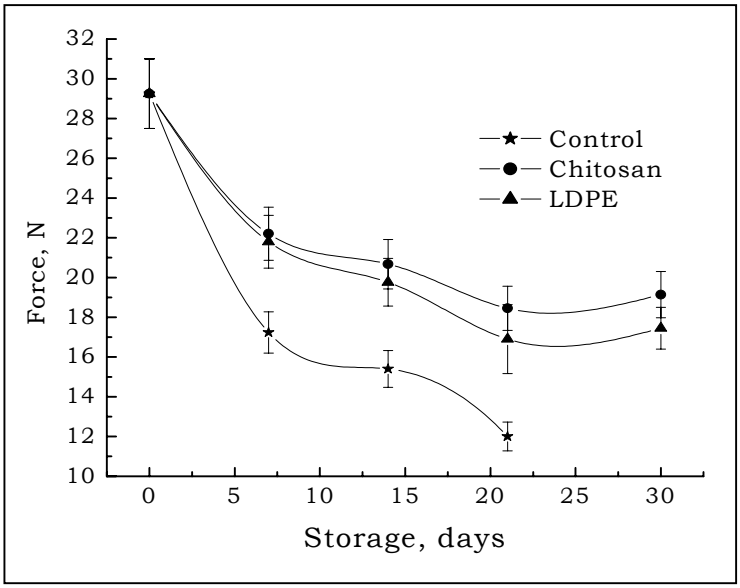

Fig. 4.19. Textural changes in penetration force of control and packaged tomatoes during storage at 27 ± 1 °C

fruits were shriveled, wilted and penetration was observed to be 12 N, whereas in MAP it was 18.5 and 17N for chitosan and LDPE packaged fruits, respectively. Beyond this period MAP stored tomatoes showed only marginal

changes for both chitosan and LDPE packaged fruits. Nevertheless, the actual values were slightly higher for chitosan-packaged fruits.

In bell pepper the texture studies (Fig. 4.20 a, b and c) were carried out under three modes such as, penetration, compression and WB shear

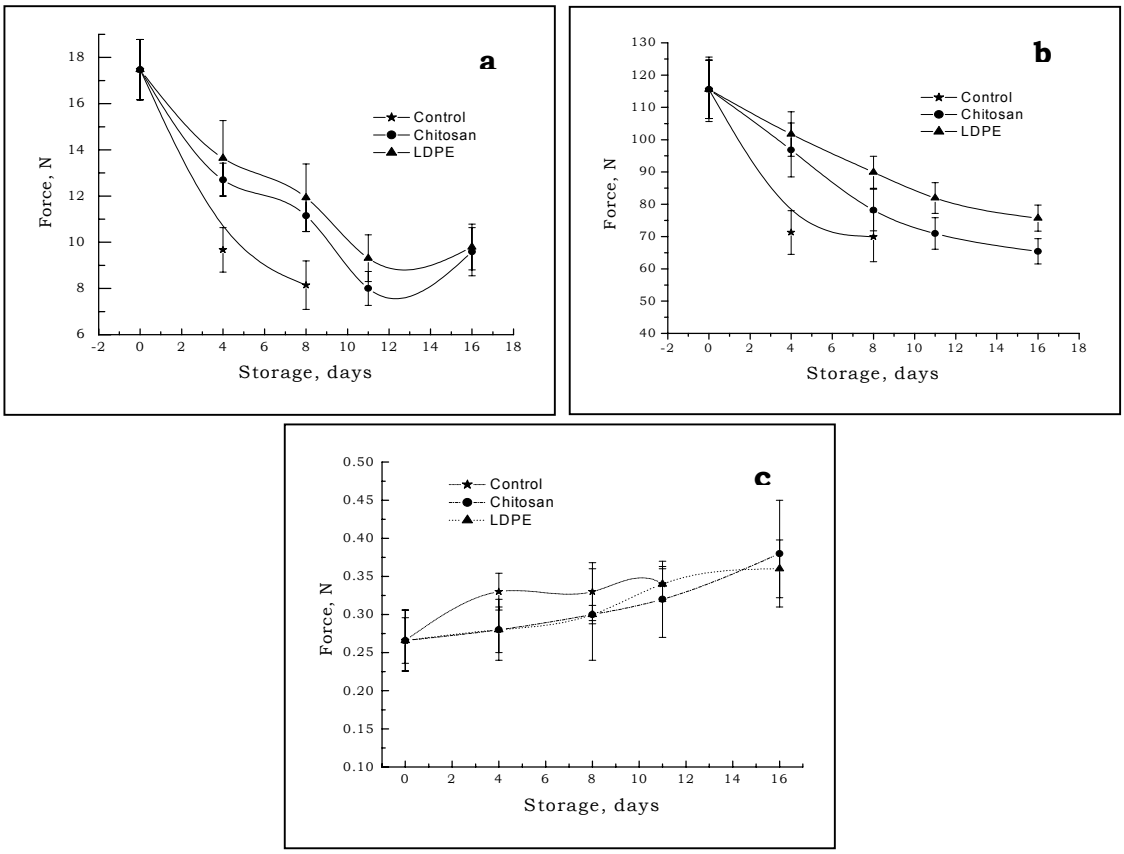

Fig. 4.20. Textural changes in penetration (a), compression (b) and shear force (c) of control and packaged bell pepper during storage at 27 ± 1 °C

(force required to cut the fruits) and compared. By 8 days of storage the unpackaged pods showed a low value of 8 N for compression (Fig.4.20a) compared to 10 and 11.5 N for LDPE and chitosan packaged pods, respectively. By 16 days of storage, unpackaged pods showed greater shriveling and loss of firmness, while LDPE and chitosan packaged pods showed comparable values. During 11 days of storage chitosan film covered pods showed a very low value, which may be due to inherent differences

associated with state of maturity of the pod at the time of harvest and difficulties in assorting the market samples.

Compression of pods (Fig.4.20b) showed a decrease in the force required during storage period. The MAP exerts considerable effects in preventing the loss of firmness. The result showed the firmness value was higher by 8 days under MAP with 80-82 N. The firmness values were approximately 70 N (for both LDPE and chitosan packaged pods) at the end of storage period with over-all texture loss of 30 to 40% by 16 days of storage.

WB shear force showed an increase in force required during the storage period (Fig.4.20c), although the penetration force decreased. As storage period increases the pods exhibited flaccidity, shriveling and wilting. The results indicated that an increase in the force required is associated with loss of water from the pods, which leads to loss of firmness.

Chemical analysis

The changes in chemical constituents during storage of tomato are shown in Table 4.10. Changes in the level of pH give an indication of the ripeness of fruits in general. However, in the present studies the pH showed no significant differences, values ranged from 4.24-4.47. This indicated pH independence of the ripening process. The TSS of tomato increased during the ripening process. Under unpackaged condition the TSS increased from 3.8 brix in greenish yellow stage to 5.4 brix in the red colour stage. In MAP stored conditions it was observed that there was a delay in ripening of tomato as indicated by slower rate of change of TSS, where it marginally increased to ~5.2 brix. Throughout the storage period the TS level showed an increasing trend, whereas the reducing sugar level decreased. The initial levels of TS and RS were 7.46 and 1.36 mg/g of tomato, respectively.

Table 4.10. Changes in pH, reducing sugar (RS) and total sugar (TS) of tomatoes during storage period

	Days	Control	Chitosan	LDPE
pH	0	4.24 ± .04	---	---
	7	4.46 ± 0.1	4.35 ± 0.08	4.47 ± 0.01
	14	4.37 ± 0.1	4.43 ± 0.02	4.46 ± 0.02
	21	4.37 ± 0.07	4.23 ± 0.03	4.23 ± 0.02
	30	-	4.46 ± 0.03	4.4 ± 0.03
TS	0	7.46 ± 1.43	---	---
	7	10.48 ± 1.2	10.48 ± 1.2	8.98 ± 0.45
	14	13.06 ± 0.98	10.89 ± 0.96	10.84 ± 0.65
	21	13.54 ± 0.86	12.14 ± 1.2	9.55 ± 0.32
	30	-	13.06 ± 1.02	9.43 ± 0.23
RS	0	1.36 ± 0.28		
	7	1.25 ± 0.21	1.17 ± 0.12	1.2 ± 0.1
	14	1.18 ± 0.34	0.98 ± 0.23	0.83 ± 0.23
	21	1.06 ± 0.23	0.87 ± 0.18	0.78 ± 0.32
	30		0.8 ± 0.16	0.75 ± 0.12
TSS	0	3.8 ± 0.2	----	---
	7	4.6 ± 0.4	4 ± 0.2	4.2 ± 0.2
	14	5.2 ± 0.4	4.6 ± 0.2	4.4 ± 0.4
	21	5.4 ± 0.4	5 ± 0.2	4.8 ± 0.2
	30	-	5.2 ± 0.2	5 ± 0.2

Unpackaged fruits gave a maximum TS level after 21 days of storage (13.1 mg/g of tomato), whereas chitosan covered fruits had values of 12.0-13.0 mg/g and those of LDPE having the least values (9-9.43 mg/g), indicating that the ripening of fruit is not identical under these conditions. Similar trend of results was observed with reducing sugar values. In bell pepper, pH showed a slight decreased value (6.8 to 6.3) and TSS did not show significant change, where the values were ranging from 4.8 to 5.2 brix under different storage conditions.

Sensory analysis of tomato and bell pepper

The PCA plot of sensory scores of tomato is shown in Fig.4.21. The axis PC1 accounted for 49% of the variance, PC2 for 18% of variance and

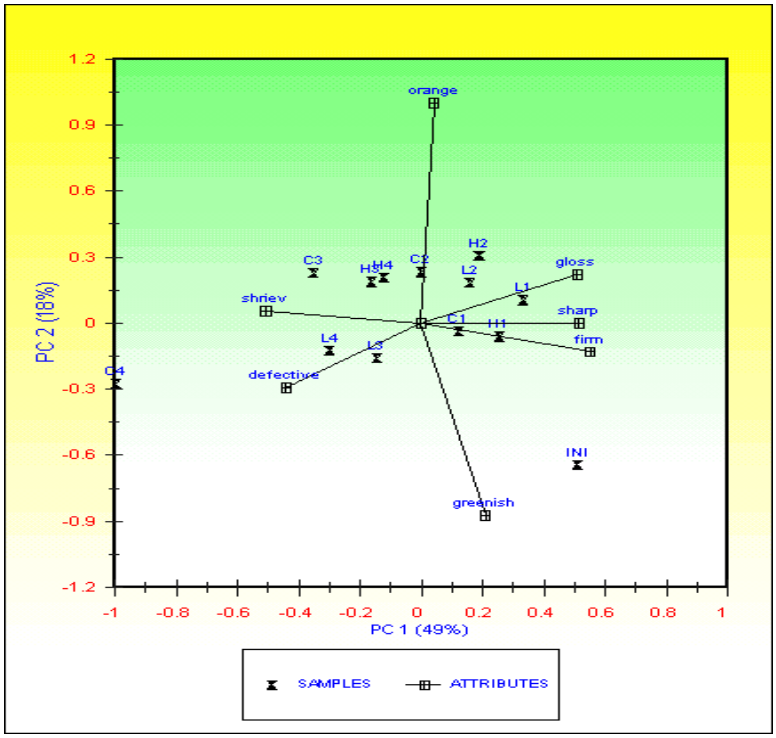

Fig. 4.21. PCA plots of tomato sensory attributes during storage

PC3 (in figure not shown) accounted for 11% of the observed variance, together they accounted for 78% of the total variance observed. The desirable notes like firmness, sharpness of cut surface, surface gloss and colour were positively loaded, while shriveling and formation of defective spots were negatively loaded in PC1. Surface gloss and sharpness and firmness were segregated and oppositely loaded to shriveling. In PC2, greenish colour was negatively loaded and orange colour positively loaded. Other attributes had lower positive and negative values. However, shriveling was positively loaded, while, development of defective spots was negatively loaded. It is seen that the tomatoes stored under different conditions occupy different quadrants indicating that they differ in their quality attributes. Initial samples were significantly differing from others because of greenness in colour. Fruits retained the desirable quality attributes upto 2nd withdrawal (15 days). Under 3rd and 4th withdrawals at all conditions they indicated the development of orange colour. By 3rd withdrawal (21 days)

control and chitosan covered samples showed shriveling while those stored with LDPE films developed defective spots. By 4th withdrawal (30 days) unpackaged fruits were spoiled while those packaged with LDPE films showed a loss of surface gloss, firmness and sharpness of cut surface, and developed more of defective spots while those packaged with chitosan films retained the desirable quality attributes indicating the beneficial role of chitosan films for extending the shelflife of tomatoes even upto 30 days. The mean scores were similar to those stored upto 21 days. Fruits packaged with LDPE films were significantly different from those unpackaged, and therefore positioned away from sharpness, glossy and firmness after 14-days of storage. MAP tomatoes were exhibiting similar trend whereas unpackaged fruits were further away from desirable quality. After 21 days of storage the unpackaged fruits exhibited more shriveling and defective spots. LDPE packaged fruits were also away from gloss, sharpness, firmness, but nearer to the defective spots showing undesirable quality. At the end of the storage period, chitosan covered tomatoes were slightly shriveled, with desirable colour. The LDPE and chitosan film covered fruits also differed considerably in that the latter were positioned positively close to desirable quality attributes. During storage for beyond 30 days, the fruits became less suitable for evaluation.

The PCA plot of sensory scores of bell pepper is shown in Fig. 4.22. The PC1 accounted for 68% of the variance, PC2 for 19% of the variance and PC3 accounted for 12 % of variance all together they account for 99% of the observed variance. In PC1, firmness, surface gloss, sharpness of edge and green colour were positively loaded, while undesirable qualities such as shriveling, development of defective spots, loss of odor and any other (representing the appearance of reddish/purplish streak) were negatively loaded. In PC2, firmness, surface gloss, sharpness of edge, were positively loaded, while shriveling, defective spots and green colour were negatively loaded.

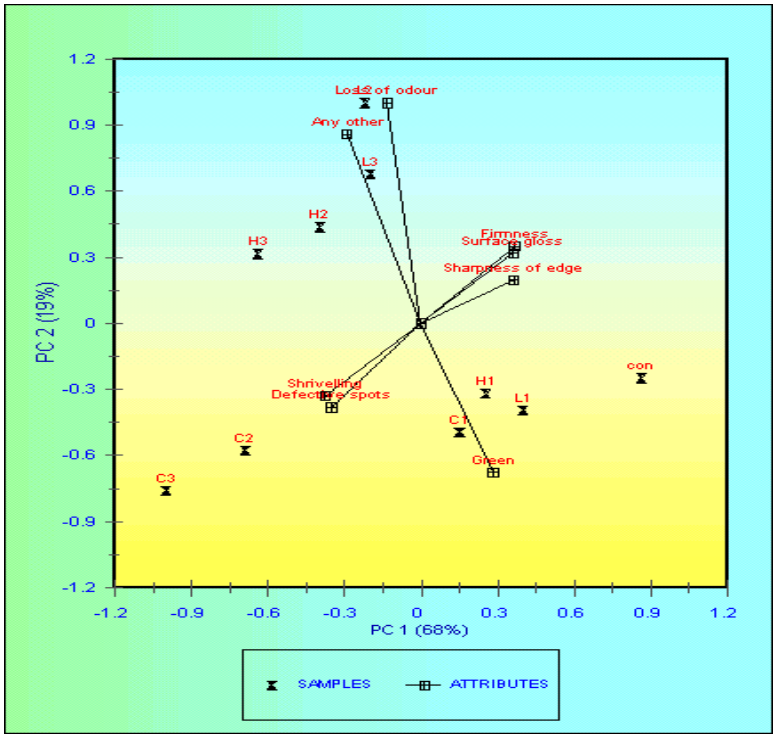

Fig.4.22. PCA plots of bell pepper sensory attributes during storage

It is seen from Fig.4.22 that bell pepper stored under different conditions occupy different quadrants indicating that they differ in their sensory quality attributes. During the storage period the pods loose firmness due to transpiration and shriveling, and defective spots also appear. In first withdrawal, under all conditions of storage pods were green in colour, and loss of firmness was not observed. All the pods were free from any loss of odour. In 2nd withdrawal control pods showed some shriveling and development of defective spots, while MAP pods were observed to retain firmness, sharpness of cut surface and retention of bell pepper odour, and surface gloss. Loss of typical bell pepper odor was noted in LDPE packaged bell pepper pods. In 3rd withdrawal also a similar trend was observed with MAP pods retaining firmness and sharpness of edge compared to unpackaged pods. The unpackaged pods showed loss of firmness and shriveling and development of defective spots was also observed.

PCA for physical and sensory attributes

Data related to changes in both physical and sensory attributes of tomato fruits are shown in Fig.4.23. The axis PC1 accounts for 50% of the variance and PC2 for 21% of variance, and PC3 (not shown) for 11% of variance and

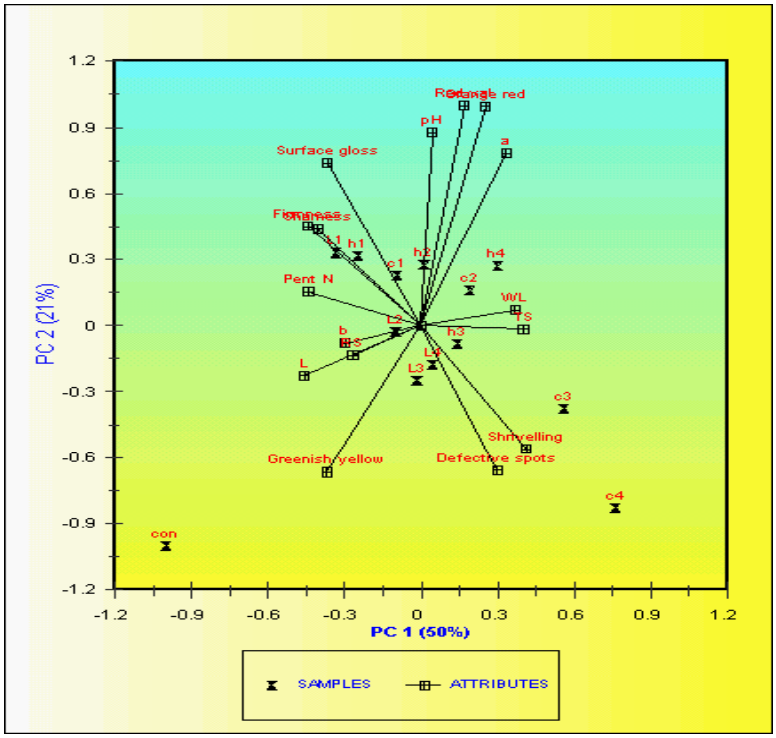

Fig. 4.23. PCA plots of physical and sensory attributes of tomatoes during storage

together they account for 82% of the total observed variance. Among the sensory attributes the undesirable notes such as appearance of defective spots and shriveling had positive value in PC1 and lower negative value in PC2. The colour change related parameters such as instrumental redness and sensory perceived greenish yellow and red colour, pH and reducing sugar were oppositely loaded in PC1 and PC2, indicating good correlation between instrumental and sensory profiling and between oppositely associated attributes. Penetration, surface gloss and sharpness of cut surface were positively loaded in PC2 and shriveling and appearance of

defective were negatively loaded in PC1. The loadings also indicate the differential segregation of desirable and undesirable attributes.

It is seen that in PC1 and PC2, tomato has negative value with greenish yellow colour and as it ripens formation of red colour was observed, which matches with instrumental ('a') value. Fruits stored under any condition followed this pattern during ripening. The relative difference exhibited by the fruits during storage is indicated by the relative location of the samples in different quadrants in PC1 and PC2. The unpackaged tomatoes, which are in equilibrium with the atmospheric gas pressure had negative loading and differed from those exposed to modified atmosphere, which had positive loading. The total sugar value, which increases with ripening are associated with the fruits stored for longer duration. The fruits stored with LDPE film had higher firmness and RS as compared to unpackaged fruits. The distribution of sample location with respect to attributes clearly highlight the advantages of chitosan based film during storage in extending the shelflife of tomato, as they retained the desirable quality attributes for a relatively longer period compared to unpackaged fruits showing about 25-30% decay and about 35-45% spoilage loss of fruits stored with LDPE films after 30 days of storage. However, the decay was very low (<10%) in case of fruits stored with chitosan film.

The PCA plot of physical and sensory attributes of bell pepper shown in Fig. 4.24 showed that the axis PC1 accounts for 52% of the variance, PC2 for 16% of variance, and PC3 (not shown in figure) observed with 12% of variance, together they account for 80% of the total observed variance. Among the sensory attributes most of the desirable notes like penetration, gloss, firmness, sharpness of cut edge, surface gloss are positively loaded in PC1, while defective spots, weight loss, shriveling, loss of odour (typical bell pepper odour) are negatively loaded. In PC2, shriveling and loss of odour have lower negative value, while sharpness of cut edge, surface gloss, instrumental gloss, green colour and pH are positively loaded.

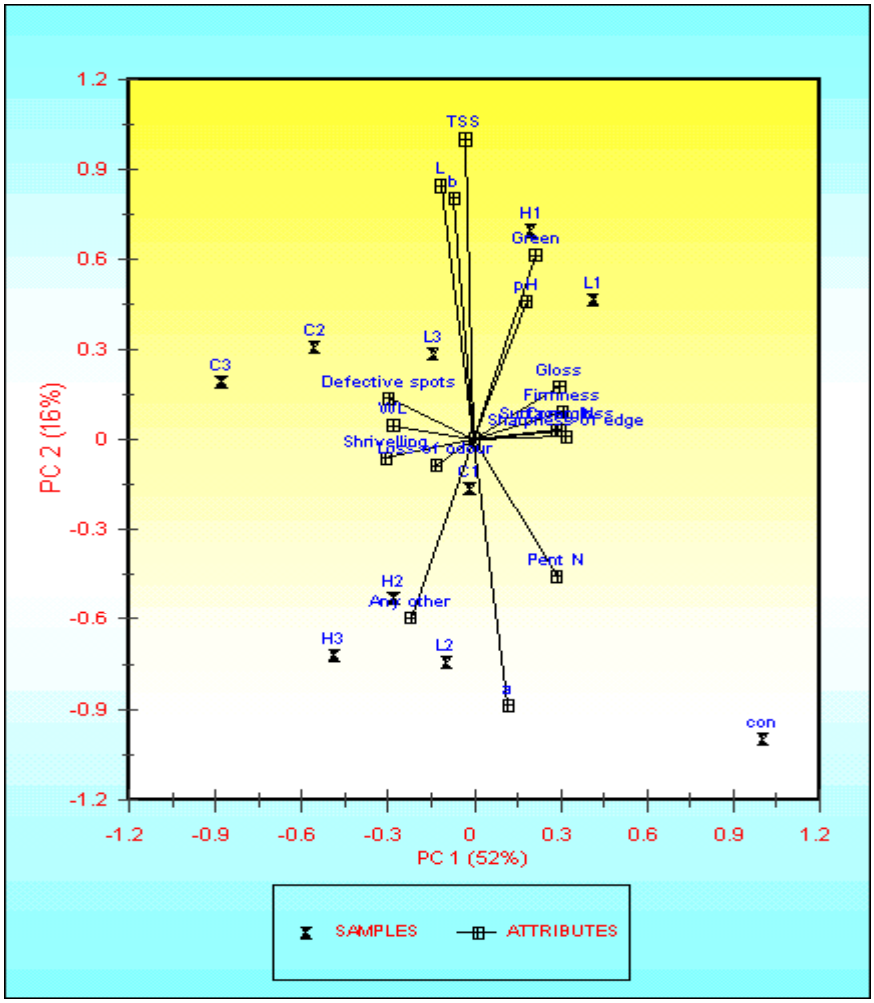

Fig. 4.24. PCA plots of physical and sensory attributes of bell pepper during storage

The formation of reddish-purplish streak and green colour, pH and sugar were oppositely loaded in PC2, indicating good correlation between instrumental values and sensory scores and between oppositely associated attributes. The loadings also indicate the differential segregation of desirable and undesirable attributes during different conditions of packaging and storage.

Storages studies on other vegetables

Storage studies on chitosan film covered okra (*Hibiscus esculentus)*, beans (*Phaseolus vulgaris*) and bell pepper (*Capsicum annaum)* showed

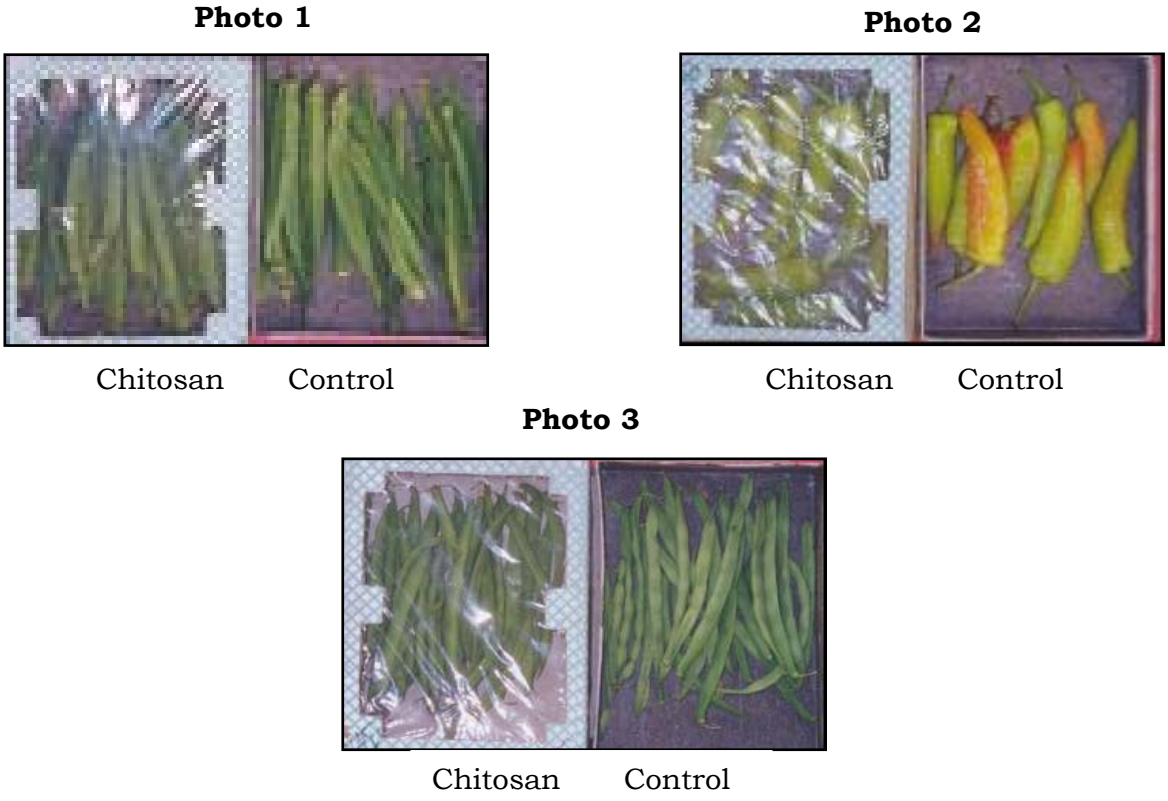

Fig.4.25. Vegetables stored for different periods (Photo 1 – okra (*Hibiscus esculentus)*, Photo 2 – bell pepper (*Capsicum annum),* Photo 3- beans (*phaseolus vulgaris*)

considerable reduction in physiological loss in weight (PLW), shriveling and colour development. The results showed shelflife extension of up to 8 days compared to 4-5 days in control. These results indicated chitosan film as a novel packaging material unit for commercial exploitation.

Conclusions

Modified atmosphere packaged conditions can extend the shelflife of tomato and bell pepper, which is beneficial for sustainable fluctuating

market availability associated with limited and seasonal availability. No differences were observed in headspace gas levels during storage period, while greater changes were observed in colour development and its retention. Changes in chemical parameters of stored fruits were very marginal. Sensory profiling indicates that synthetic film packaged fruits exhibited loss of typical aroma, while chitosan packaged fruits retained it. Firmness and development of desirable red color and retention of green colour are the major factors in price and market value of tomatoes and bell pepper. The unpackaged fruits showed decaying symptoms at an early stage than the packaged samples. The decay pattern indicated beneficial role of chitosan films for extending the shelflife of bell pepper. However, desirable quality attributes were retained for longer periods with chitosan film packaged samples as compared to LDPE film packaged fruits, indicating the greater application potential of chitosan films and associated with their biodegradable eco-friendly nature.

C. Dairy products (*Peda*)

The colour of *peda* measured instrumentally did not show greater changes, the hue angle θ varied from 61.3–62.8^{o}. Different packaging conditions showed considerable effect on the quality of *peda* (Table 4.11). The textural force (penetration, N) increased during storage period. On 4th day of storage chitosan and chitosan coated butter film packaged *peda* showed higher force of 8.2 and 8.1 N., respectively. The latter retained the force upto 12 days of storage, whereas control showed a higher value of 8.9 N at the end of the storage period. No significant difference was observed in moisture content and acidity values. The moisture content slightly decreased from 16.2 to15.7 %. The acidity value increased from 0.25 to 0.31 % and least acidity increase was observed (0.29 %) in *peda* stored in chitosan coated butter paper.

Table 4.11. Changes in physico-chemical and microbiological quality of *peda* during storage under different conditions

Conditions	Force (N)	Moisture (%)	Acidity (%)	SPC Log $_{10}$cfu/g	Col	Y&M
Initial	7.8	16.2	0.25	15	<10	<10
4 Days						
Control	7.9	16.1	0.27	300	<10	<10
Butter Paper	7.9	16.1	0.27	320	<10	<10
Chitosan film	8.2	15.9	0.28	17	<10	<10
Chitosan coated-butter paper sheet	8.0	16.1	0.27	20	<10	<10
8 Days						
Control	8.3	16.0	0.29	63×10^4	<10	70
Butter Paper	8.2	16.0	0.28	75×10^4	2.78	<10
Chitosan film	8.4	15.8	0.30	60×10^1	<10	20
Chitosan coated-butter paper sheet	8.0	16.0	0.27	2×10^3	<10	<20
12 Days						
Control	8.9	15.9	0.31	$>10^6$	80	600
Butter Paper	8.4	15.8	0.31	$>10^6$	80	700
Chitosan film	8.6	15.7	0.32	1.5×10^3	48	<300
Chitosan coated-butter paper sheet	8.1	15.9	0.29	3.2×10^3	68	<300

SPC=standard plate count, cfu/g, Col =coliforms, Y & M = yeasts and moulds

Spoilage of milk products occurs due to microorganisms. During the storage period the SPC count increased from 1 to 6 log cfu/g of control and LDPE stored *peda* sample, with an increase to 2 log cfu/g during the first 4 days, whereas in chitosan and chitosan coated butter paper packaged *peda*, no increase in colony count was observed. But at the end of 8 days of storage it was increased to 5 log cfu/g in control and butter paper packaged *peda*, while in chitosan coated butter paper *peda* sample it was 3 log cfu/g and least was observed in chitosan film packaged *peda* (2 log cfu /g). But at the end of storage period SPC was 6 log cfu/g in control and butter paper packaged *peda*, whereas in chitosan film and chitosan coated butter paper *peda* they were 3 log cfu/g. Coliforms did not show much increase during storage period. A similar trend was observed in yeast and mold counts. At

the end of the storage period the highest count was observed in control, least (2 log cfu/g) in chitosan film packed *peda*. Microbiologically control samples packaged in butter paper covered carton showed multiplication of mesophlic aerobes with higher SPC counts beyond 4 days of storage, whereas samples packaged in chitosan and chitosan coated butter paper sheet cartons remained acceptable even after 12 days of storage as their SPC counts were with in acceptable range (<50,000 cfu/g).

Sensory analysis of stored *peda*

During the storage period no significant colour difference was observed by surface colour observations, as the values ranged between 6.9-7.2. The textural force (finger feel), the main factor for quality changes observed is due changes in moisture content of the sample, which increased during storage. It is observed from Table 4.12 that the milky note decreases during the storage period. The initial value was around 9.6-9.8, and it decreased to 6.5-7.8 during storage. Chitosan coated butter paper showed higher retention of milky note compared to other packaged conditions even upto 12 days. Chemical note also showed a similar trend. Sourness is due to conversion of lactose to lactic acid, by the microorganisms, which converted, which increased during storage. On 12th day, the samples were associated with highest sourness value, but least was observed in chitosan coated butter paper (1.0). Sensory results showed that, initial *peda* sample had high overall quality (OQ) value of 9.7-9.8. On 4th day, no significant differences was observed in OQ of the *peda* samples under all packaging conditions. On 8th day of storage, with control and chitosan film, the values showed a slight decrease, probably due to increase in staleness and chemical note, respectively. Between chitosan film and chitosan coated butter paper covered cartons, although microbial load of the *peda* samples was comparable, chemical and sour notes were perceived even after 8 and 12 days of storage and hence OQ was much lower with chitosan film. These indicated that only samples stored in chitosan coated butter paper covered cartons retained the

desirable quality attributes for a longer period compared to other conditions of storage.

Table 4.12. Sensory quality of *peda* during storage under different conditions

Conditions	Textural Force	Milky	Chem	Sour	Stale	OQ
Initial	4.5b	9.8a	0.7a	0.7a	0a	9.8a
4 Days						
Control	4.8b	8.4a	0.9a	1.2a	1.2a	9.5a
Butter Paper	4.9b	8.6a	0.8a	1.0a	1.2a	9.4a
Chitosan film	5.1b	7.8b	1.8b	1.7a	1.0a	8.8a
Chitosan coated-butter paper sheet	4.6b	8.8a	0.8a	1.1a	0.5a	9.8a
8 Days						
Control	3.2a	8.3b	1.0a	1.6b	4.2b	7.5b
Butter Paper	3.5a	6.3c	0.8a	0.8a	1.2a	8.9a
Chitosan film	5.9c	6.8c	2.7b	3.2c	1.3a	7.8b
Chitosan coated-butter paper sheet	4.7b	8.5a	0.8a	1.0a	1.0a	9.3a
12 Days						
Control	6.5c	6.5c	1.5ab	ND	5.6c	ND
Butter Paper	6.3c	6,7c	1.3a	ND	5.3bc	ND
Chitosan film	6.2c	6.3c	3.1bc	3.6c	1.6a	7.6b
Chitosan-coated butter paper sheet	5.0b	7.8b	0.8a	1.0a	1.5a	8.6a

Mean with same letter in a column did not differ significantly ($p \leq 0.05$) DMRT; Tex F= Texture (finger feel), Chem = chemical, OQ= overall quality, ND =not determined

D. Bakery products

Wheat flour sample selected for the studies had 0.5 % ash, 10.5 % dry gluten, 60 ml SDS- Sedimentation value, 360 falling number, 10.2 % damaged starch and 62.2 % farinograph water absorption, 4.0 min stability and 52 calorimeter value. The data indicated that wheat flour used for the study was of medium strength and typical of Indian flours.

Storage characteristics of bar cake

Bar cakes packed using chitosan film and chitosan coated butter paper

The effect of bar cakes, containing 0.5 % calcium propionate and 0.2 % acetic acid and packed using chitosan film (CF) or butter paper coated with chitosan (BF) or polypropylene (PF) is shown in Fig. 4.26. The

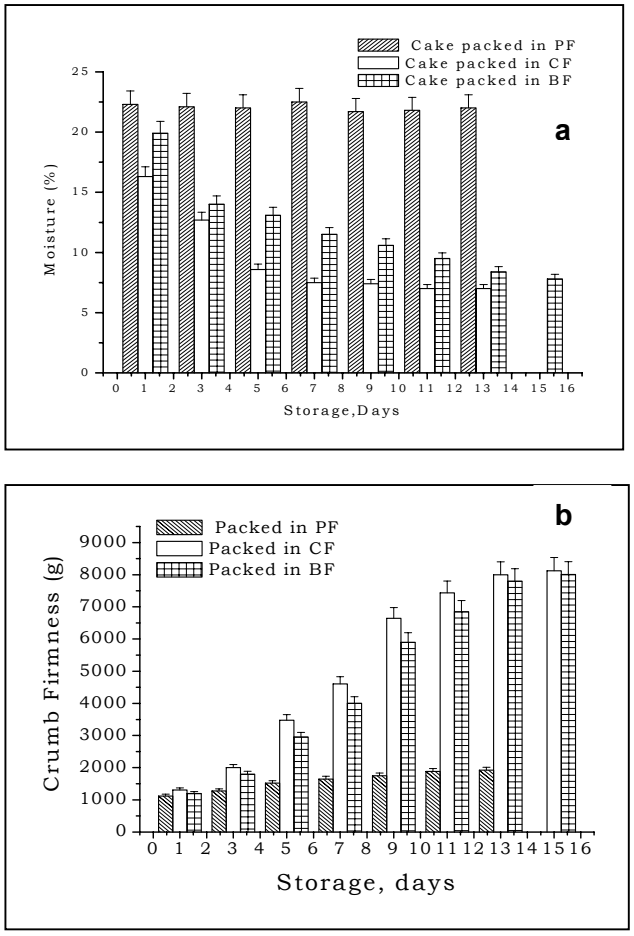

Fig. 4.26. The moisture (%) (a) and crumb firmness (b) of bar cake under different packaged conditions during storage

result showed not much change in the moisture values during storage of bar cake packed in polypropylene. However, bar cakes stored in chitosan film or butter paper coated chitosan showed too much loss of moisture. On first day

their moisture content was 16.3 % and 19.9% as against 22.3 % in control. The moisture content further decreased to 7 % and 8.4% by 13th day and showed not much change thereafter upto fifteen days of storage period. From first day to thirteenth day the crumb firmness value of control cake packed in polypropylene increased from 1120 to 1920 g and that packed in chitosan film increased from 1305 to 8001 g. and in butter paper coated with chitosan it was 1200 to 7800g (Fig.4.26). This indicates that the cakes packed in chitosan film are very much harder than control bar cake initially as well as during storage. However the bar cakes packed in butter paper coated with chitosan were free of mold growth upto 15 days of storage period when compared to control which showed mold growth at fifteenth day. These data indicate that packing of cakes using either chitosan film or butter paper coated with chitosan is not beneficial in extending the shelflife of cakes. Hence an attempt was made to incorporate chitosan into the dough and to study the quality changes in the product.

(ii). Effect of combination of chitosan (0.1 and 0.2 %), calcium propionate (0.5 %) and acetic acid (0.2 %) on storage and sensory characteristics of bar cake packed in polypropylene

The crumb firmness data of control cake prepared with 0.5 % calcium propionate and 0.2 % acetic acid (A) and cakes prepared with 0.1 % chitosan (B) or 0.2 % chitosan (C) in combination with 0.5 % calcium propionate and 0.2 % acetic acid in mixing stage of batter and packed in polypropylene are presented in Fig. 4.27. With increase in storage period from first to 13th day the crumb firmness value increased from 1150 to 1930 g for A, 1120 to 1935 g for B, and 1100 to 1950g for C. This indicates that there was noticeable increase in the crumb firmness values during storage upto 13th day. Appearance of mold growth was at 15th day and for A and B cakes

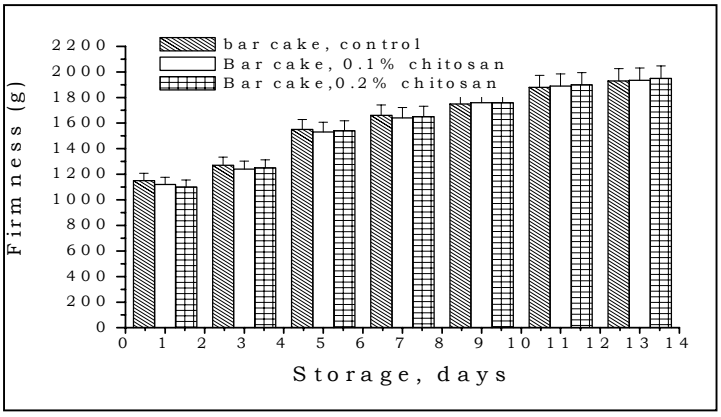

Fig. 4.27. The crumb firmness of bar cake under different packaged conditions during storage

there were no appearance of mold growth upto 15th day for C bar cake. The sensory characteristics showed (Table.4.13) not much change in weight, volume and specific volume. All stored bar cakes possessed golden brown crust colour, creamish crumb colour and fine crumb grain with thin cell walls. The cakes were soft. The eating quality of A, B and C cakes were typical

Table 4.13. Quality characteristics of bar cake with chitosan and packed in polypropylene

Parameters	A	B	C
Weight (g)	370	371	372
Volume (ml)	1250	1255	1260
Specific volume (ml / g)	3.38	3.38	3.39
Crumb firmness (force, g)	1150	1120	1100
Crust			
Colour	Golden brown	Golden brown	Golden brown
Shape	Normal	Normal	Normal
Crumb			
Colour	Creamish	Creamish	Creamish
Grain	Fine, uniform	Fine, uniform	Fine, uniform
Eating quality	Typical	Typical	Typical

Storage of plain bread

Effect of moisture content and crumb firmness of plain bread prepared using 0.3% calcium propionate, 0.1% acetic acid, packed in polypropylene (A), chitosan film (B) and butter paper coated with chitosan (C) is presented in Fig 4.28. The results showed a decrease in moisture content during

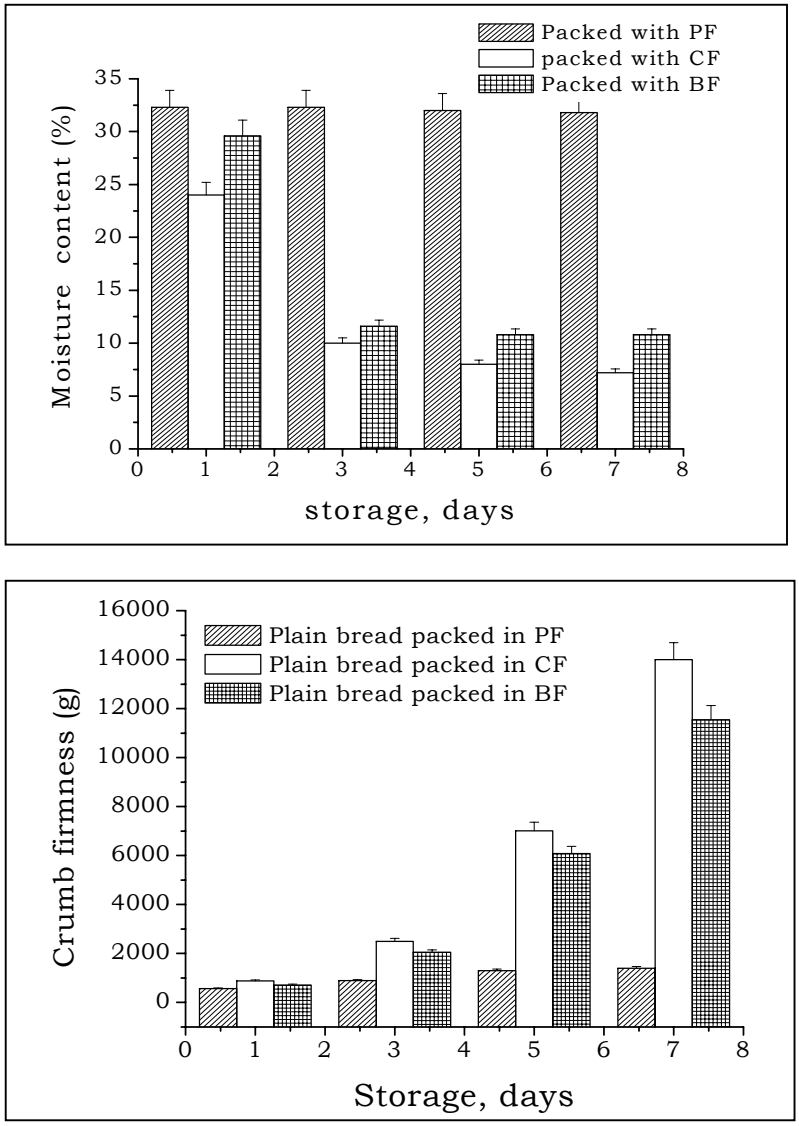

Fig. 4.28. Moisture content and crumb firmness of plain bread packed in polypropylene (PF), chitosan film (CF) and chitosan coated butter paper (BF)

storage upto 7 days from 32.3 to 31.8 % for A bread, 24 to 7.2 % for B bread 29.6 to 10.8% for C bread. During storage of bread from 1 to 7 days the crumb firmness value increased from 565 to 1400 g for A bread and 880 to 14000 g for B bread. 710 to 11550 g for C bread. It can be inferred from the above data that there was too much loss of moisture for the B and C bread packed in chitosan film and butter paper coated chitosan. The mold growth appeared on 8th day for A bread and there was no mold growth for B and C breads.

(iii). Effect of combination of chitosan (0.1 and 0.2%), calcium propionate (0.3%) and acetic acid (0.1%) on firmness and sensory characteristics of plain bread packed in polypropylene

The effect of addition of 0.1% chitosan (A) or 0.2 % chitosan (B) in combination with 0.3% calcium propionate, 0.1% acetic acid in dough stage and bread packed in polypropylene is presented in Table 4.14. During

Table 4.14. Storage characteristics of plain bread with chitosan and packed in polypropylene

Days	Moisture (%)		Crumb firmness (Force, g)		Appearance of mold growth	
	A	B	A	B	A	B
1	32.5	32.1	560	560	_	_
3	32.0	32.9	880	885	_	_
5	32.2	32.4	1290	1300	_	_
7	32.0	32.5	1410	1395	_	_
8	_	_	_	_	+	+

storage, there was not much change in the moisture content of A and B breads. The crumb firmness value increased from 560 to 1410 g for A bread and from 560 to 1395 g for B bread. The control showed a value of 1400 at

the end of storage period. Appearance of mold growth was on 8th day for both A and B breads indicating no improvement in the shelflife of breads prepared using of 0.1 or 0.2 % chitosan in combination with 0.3 % calcium propionate and 0.1 % acetic acid.

The sensory evaluation result showed not much change in weight, volume and specific volume (Table 4.15). All the breads possessed golden brown crust colour, normal crust shape, creamish white crumb colour and medium fine crumb grain. The breads were soft as indicated by the crumb firmness value of 555 – 560 g. There was no foreign taste in the breads.

Table 4.15. Quality characteristics of plain bread with chitosan and packed in polypropylene

Parameters		A	B	C
Weight (g)		401	400	401
Volume (ml)		1605	1610	1600
Specific volume (ml / g)		4.0	4.03	3.99
Crumb firmness (g)		560	560	555
Crust	Colour	Golden brown	Golden brown	Golden brown
	Shape	Normal	Normal	Normal
Crumb	Colour	Creamish white	Creamish white	Creamish white
	Grain	Medium fine uniform	Medium fine uniform	Medium fine uniform
	Mouthfeel	Easy breakdown	Easy breakdown	Easy breakdown
	Taste	Typical	Typical	Typical

(iv). Effect of combination of chitosan (0.1 and 0.2%), calcium propionate (0.5%) and acetic acid (0.2%) on storage and sensory characteristics of plain bread packed in polypropylene

The effect of addition of 0.1 % chitosan (B) or 0.2 % chitosan (C) in combination with 0.5 % calcium propionate, 0.2 % acetic acid as against

control with calcium propionate (0.5 %), acetic acid (0.2 %) (A) in dough stage on moisture content and crumb firmness of bread packed in polypropylene is presented in Fig.4.29. During storage, there was not much

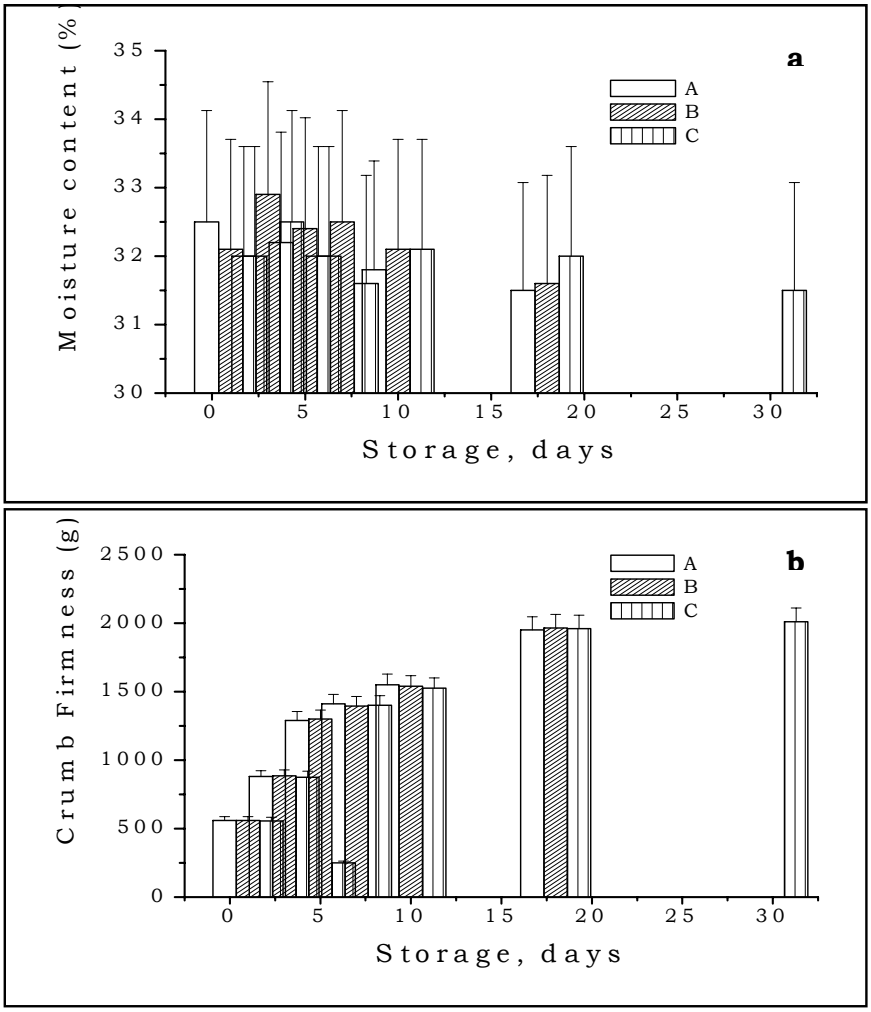

Fig.4.29 The percent moisture (a) and crumb firmness (b) of plain bread A: control; B: 0.1 % chitosan added into dough; C: 0.2 % chitosan added into dough

change in the moisture content of A, B and C plain breads. The increase in crumb firmness value of A, B and C plain breads was similar. Appearance of mold growth was at 18th day for both A and B breads and there was no mold growth even at 13th day for C bread. It denoted that combination of 0.1 % chitosan, 0.5 % calcium propionate, 0.2 % acetic acid in dough stage did not

improve the shelflife, while combination of 0.2 % chitosan, 0.5 % calcium propionate, 0.2 % acetic acid showed improvement in shelflife of plain bread.

The sensory evaluation of plain bread showed no change in weight, volume and specific volume (Table.4.16). All the stored breads possessed golden brown crust colour, normal crust shape, creamish white crumb colour and medium fine crumb grain. The breads were soft as indicated by the crumb firmness value of 555 – 560 g. All the breads had strong acetic acid smell.

Table 4.16. Quality characteristics of plain bread with chitosan and packed in polypropylene

Parameters		A	B	C
Weight (g)		401	400	401
Volume (ml)		1605	1610	1600
Specific volume (ml / g)		4.00	4.03	3.99
Crumb firmness (g)		560	560	555
Crust	Colour	Golden brown	Golden brown	Golden brown
	Shape	Normal	Normal	Normal
Crumb	Colour	Creamish white	Creamish white	Creamish white
	Grain	Medium fine uniform	Medium fine uniform	Medium fine uniform
	Mouthfeel	Easy breakdown	Easy breakdown	Easy breakdown
	Taste	Acetic acid smell	Acetic acid smell	Acetic acid smell

(v). Effect of combination of chitosan (0.2 %), calcium propionate (0.5 %) and acetic acid (0.2 %) on storage and sensory characteristics of sweet bread packed in polypropylene

The effect of addition of 0.2 % chitosan in combination with 0.5 % calcium propionate, 0.2 % acetic acid (B) as against control with calcium

propionate (0.5 %), acetic acid (0.2 %), without chitosan (A) in dough stage and packed in polypropylene is presented in Table 4.17. During storage,

Table 4.17. Storage characteristics of sweet bread with chitosan and packed in polypropylene

Days	Moisture (%)		Crumb firmness (Force, g)		Appearance of mold growth	
	A	B	A	B	A	B
1	30.2	30.1	760	740	_	_
3	29.8	30.0	1050	1025	_	_
5	29.5	29.9	1690	1650	_	_
7	30.0	29.8	1980	1995	_	_
9	29.8	29.9	2350	2300	_	_
28	_	29.5	_	2650	+	_

there was not much change in the moisture content of A and B sweet breads. The increase in crumb firmness values of A and B breads were similar. Appearance of mold growth was at twenty eighth days for A bread and there was no mold growth even at twenty eighth day for B bread. The results showed that combination of 0.2 % chitosan, 0.5 % calcium propionate, 0.2 % acetic acid in dough stage improved the shelflife of sweet bread. However the sweet breads showed strong acetic acid smell.

The sensory evaluation results showed no change in weight, volume and specific volume (Table.4.18). All stored the breads possessed slightly dark brown crust colour, normal crust shape, creamish white crumb colour and medium fine crumb grain. The breads were soft as indicated by the crumb firmness value of 740 – 760 g. The taste of bread possess strong acetic acid smell.

Table 4.18. Quality characteristics of sweet bread with chitosan and packed in polypropylene

Parameters		**A**	**B**	**C**
Weight (g)		402	403	402
Volume (ml)		1610	1615	1615
Specific volume (ml / g)		4.00	4.01	4.02
firmness (g)		760	750	740
Crust	Colour	Slightly dark brown	Slightly dark brown	Slightly dark brown
	Shape	Normal	Normal	Normal
Crumb	Colour	Creamish white	Creamish white	Creamish white
	Grain	Medium fine uniform	Medium fine uniform	Medium fine uniform
	Mouthfeel	Easy breakdown	Easy breakdown	Easy breakdown
	Taste	Acetic acid smell	Acetic acid smell	Acetic acid smell

Conclusions

Use of chitosan coated butter paper for packaging eliminated imparting any acidic note to the *peda* during storage, as direct contact with the stored material did not occur. The chitosan coated butter paper coverage for the lid possesses limited reusability. Suitable sized packaging boxes could be designed and used for wholesale or retail trading of *peda* and other diary products such as *burfi* or flavored *burfi*, which has compatibility for packaging and storage. The potential of surface coating of chitosan for shelflife extension is cost effective and could be explored for other snacks also. In Bakery products, chitosan film and chitosan coated butter paper did not show any effect on the storage characteristics. But incorporation of chitosan into the dough showed the better effect in extension of shelflife as well as retardation the growth of molds.

Introduction

Packaging is an essential requirement in the storage of processed foods, fresh horticultural produce, bakery confectionary and other multi-ingredient component food products. In general, packaging plays a key role in the growth of food processing industries. Synthetic plastics as a packaging material are losing its utility because of non-biodegradability (Kittur *et al.*, 1998). Challenges and opportunities for edible and biodegradable polymer films as an alternative to synthetic plastics is of considerable interest, as such materials have many advantages as they are replenishable and accord sufficient protection of food materials (Tharanathan, 2003). Microbial growth on the film surface may lead to spoilage and decay of food materials, but new breakthrough was possible in such films by incorporating antimicrobial compounds (Han, 2000). Consumer demand for foods without chemical preservatives has led to the discovery of new natural antimicrobial agents, which significantly inhibit the growth of various spoilage and pathogenic organisms. Any packaging material having both antimicrobial and biodegradable properties will be eco-friendly, thus reducing the environmental pollution hazards.

In recent years, antimicrobial and antifungal activities of chitosan and its degradation products such as, chitooligomers and low molecular weight chitosans have been studied by several researchers (Shahidi *et al.*, 1999; No *et al.*, 2002; Vishu Kumar *et al.*, 2004), with particular emphasis on their ability as a food preservative (Chen *et al* , 1998). The antimicrobial property of chitosan is due to the presence of free NH_2^+ groups at C-2 position, which makes it more soluble and cationic than chitin. The precise mechanism of its antimicrobial property is still not known. Wang (1992) observed that chitosan could inactivate *S. aureus* and *L. monocytogenes* at a pH 5.5 or 6.5. Chang et al. (1989) found that chitosan concentrations of >0.005% was sufficient to inactivate *S. aureus*. Only a few reports are available on the

antimicrobial property of chitosan film *per se*. Coma *et al*. (2002) studied the effect of chitosan-coated films on *L. monocytogenes*.

The main emphasis of the present work was to investigate the antimicrobial and antifungal activities of chitosan film against foodborne pathogens and spoilage organisms and to understand its effect on the structural deformations of selected bacterial isolates.

Materials and Methods

Bacterial and fungal strains

The bacterial strains used in the present study included *L. monocytogenes* Scott A, obtained through the courtesy of Dr. Arun Bhunia, Purdue university, USA; *Staphylococcus aureus* FRI 722, obtained through the courtesy of Dr. S. Notermans, Public Health Laboratory, Netherlands; *Yersinia enterocolitica* MTCC 859 as well as the fungal strains *Rhizopus* NCIM 997, *Fusarium* NCIM 104 and *Penicillium* MTCC 2007 were obtained from the Institute of Microbial Technology, Chandigarh, India. *Bacillus cereus* F4810 and *Escherichia coli* D21 were obtained from the culture collection maintained in Food Microbiology department of CFTRI.

Growth conditions

The bacterial strains were maintained on brain heart infusion agar (BHI) slants (Himedia, Mumbai, India) at 4 °C. Prior to use, the cultures were propagated twice in 10 ml BHI broth at 37 °C for 18 hr at 150 rpm, except for *Y. enterocolitica* which was grown at 32 °C for 18 hr at 150 rpm. The cells were harvested by centrifugation at 10,000 rpm for 20 min at 4 °C and resuspended in 10 ml of 0.85% of sterile saline, under aseptic condition.

The fungal strains were maintained on potato dextrose agar (PDA) slants [Himedia, Mumbai, India] and propagated in potato dextrose broth prior to use in the experiments. The inoculum was prepared by growing the

fungi on slants for 6 to 8 days. The spores were transferred from the slants with sterile distilled water to achieve the optimal spore concentration of 5 $\log_{10}$ to 6 $\log_{10}$ cfu/ml.

Antimicrobial activity of chitosan films

Screening for dilution level for broth medium study

Initially 3 $\log_{10}$ to 9 $\log_{10}$ cfu/ml levels of selected bacterial cultures were added to 9 ml of BHI broth along with surface sterilized chitosan (25 mg) films, and incubated at 37 °C (32 °C for *Y. enterocolitica*) for 24 hr and observed for difference in optical density by measuring absorbance at 600 nm along with control.

Growth curve

Individual growth curves were obtained by inoculating *S. aureus*, *L. monocytogenes* and *Y. enterocolitica*, at 3 $\log_{10}$ cfu/ml level in BHI broth along with 10 mg of surface sterilized chitosan film, and measuring OD 600 at regular intervals for 24 hr. For comparison, the specific cell growth rate during exponential growth phase was also calculated using the equation (Chung *et al.*, 2003)

$\delta x\ (t)\ \delta t = \mu\ x(t)$, where

x(t)= cell concentration in the medium OD 600

μ = specific growth rate; t =time in hr

Agar diffusion test

Plate count agar (PCA) (Himedia, Mumbai, India) plates were prepared and 100 µl of the selected dilutions of respective bacterial cultures were spread plated in duplicate. The chitosan films of 5 mm diameter were surface sterilized using absolute alcohol and rinsed in sterile 0.85% saline and were

placed over the inoculated plate. The plates were incubated at 8°C for 4 hr to enhance diffusion followed by prolonged incubation at 37 °C for 24 hr.

Broth culture assay

Individual cultures of *S. aureus, L. monocytogenes and Y. enterocolitica* were diluted in 0.85% sterile saline to achieve concentrations of 3, 4 and 5 $\log_{10}$ cfu/ml. To 9 ml of sterilized nutrient broth, 1 ml of respective cell dilutions was added along with 3, 5 and 10 ± 0.2 mg of chitosan film. The flasks were incubated in a shaker at 37 °C, 150 rpm (Remi, Mumbai, India). The cell growth was monitored periodically at 24 hr, 48 hr and 72 hr by measuring OD 600 (SECOMAM Anthelie, Advance V2.5b, France) and viable plate count. All the experiments were carried out in duplicate unless mentioned otherwise.

Antifungal activity of chitosan films

Growth repression studies on PDA plates

Individual fungal cultures were point inoculated onto PDA plate and chitosan films (5 mm diameter) were placed near inoculated point. The plates were incubated at 30°C for about 5 days to observe the growth of culture. A control plate without placing the chitosan films was also kept for comparison.

Inhibition of fungal growth in the broth system

Individual fungal cultures were inoculated in duplicates at spore counts of 3 $\log_{10}$ and 6 $\log_{10}$ cfu/ml into potato dextrose broth (20 ml) and chitosan film piece was added at levels of 20 and 100 mg. The flasks were incubated at 30 °C for 5 days and the cell growth pattern was noted periodically. The fungi growth was observed in terms of dry weight by

transferring fungal mat into petri plates and drying in an oven at 60 °C until two consecutive consistent readings were obtained.

Scanning electron microscopy (SEM)

After 24 hr incubation, 0.5 mL aliquots were transferred to micro-centrifuge tubes followed by centrifugation. The pellets obtained were treated with phosphate buffer (pH 7.0, 0.3 M), fixed with glutaraldehyde (1%) for 1 hr at 4°C and further treated with 10%- absolute alcohol in a sequential manner. The dried samples thus obtained were spread over double-sided conducting adhesive tape pasted on a metallic stub and coated with gold (100 μ) in a sputter coating unit for 5 min and observed under Scanning electron microscope (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK) at 20 kV.

Results and Discussion

Agar diffusion method

Initial study of chitosan antimicrobial activity was carried out by well agar diffusion method. It was observed that, chitosan solution was not diffused into the agar medium. The organisms were grown around the well, without any inhibition zone. It may be due to highly viscous chitosan solution. So disc agar method was done to observe antimicrobial activity of chitosan film. In disc agar diffusion method, no inhibition zones by chitosan film were observed showing its absence of diffusion into the medium as observed in well agar method. However, no growth of bacteria occurred below the chitosan disc. Later the disc was removed after 48 hr of exposure and the plates were incubated for another 24 hr to observe for any growth of bacteria in the chitosan-exposed area. The absence of growth even after 24 hr indicated that chitosan has definite lytic effect, although it failed to diffuse

into the agar medium. Coma *et al.* (2002) observed a poor inhibitory activity of the chitosan film forming solution in agar medium and the film showed no inhibition even near discs.

Screening for organisms

The minimum inhibitory effect of chitosan film (24 mg) was determined by tube assay method, by keeping sterilized films in different concentrations of culture. For the present study, cell concentration from 3 $\log_{10}$ to 7 $\log_{10}$ were taken in different test tubes (in triplicate) and kept at 37 °C for 24 hr (32 °C for *Y. enterocolitica*). The colony counts were made after 24 hr (Table 5.1).

Table 5.1. Growth of different organisms in BHI broth

Species	7 (log cfu/ml)		6 (log fu/ml		5 (log cfu/ml		4 (log cfu/ml		3 (log cfu/ml	
Incubation period (hr)	24	48	24	48	24	48	24	48	24	48
Bacillus	>9	ND	>9	ND	>9	ND	>9	ND	>9	ND
E-coli	>9	ND	>9	ND	>9	ND	>9	ND	>9	ND
S.aureus	8.4	8.8	7.12	8.2	2.45	3.42	1.65	2.3	<1	<1
Y.enterocolotica	>9	ND	>9	NG	1.45	2.43	1.12	1.39	1.2	1.78
L.monocytogenes	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

NG- No growth, ND- not determined

It was observed the 24 mg of chitosan film had a better effect against *L. monocytogenes*, but for *Bacillus* and *E. coli* it showed lesser effect, but considerable effect at lower concentration of *S. aureus* and *Y. enterocolitica.* For further detail study *L. monocytogenes, S. aureus* and *Y. enterocolitica* organisms were selected.

Growth curve of *L. monocytogenes, S. aureus* and *Y. enterocolitica*

The inhibitory effect of chitosan film on *L. monocytogenes, S. aureus* and *Y. enterocolitica* is shown in Fig.5.1. In all the organisms, growth was inhibited by chitosan film. In *L. monocytogenes*, reduced growth was observed from lag phase itself when compared with the control. Although in the lag phase the cell mass difference was not much, at exponential phase the control had 1.5 times higher cell mass than the experimental medium containing the chitosan film. Similarly, with *S. aureus*, the cell concentration in the control was 1.4 times higher than in the chitosan film. Coma *et al.*

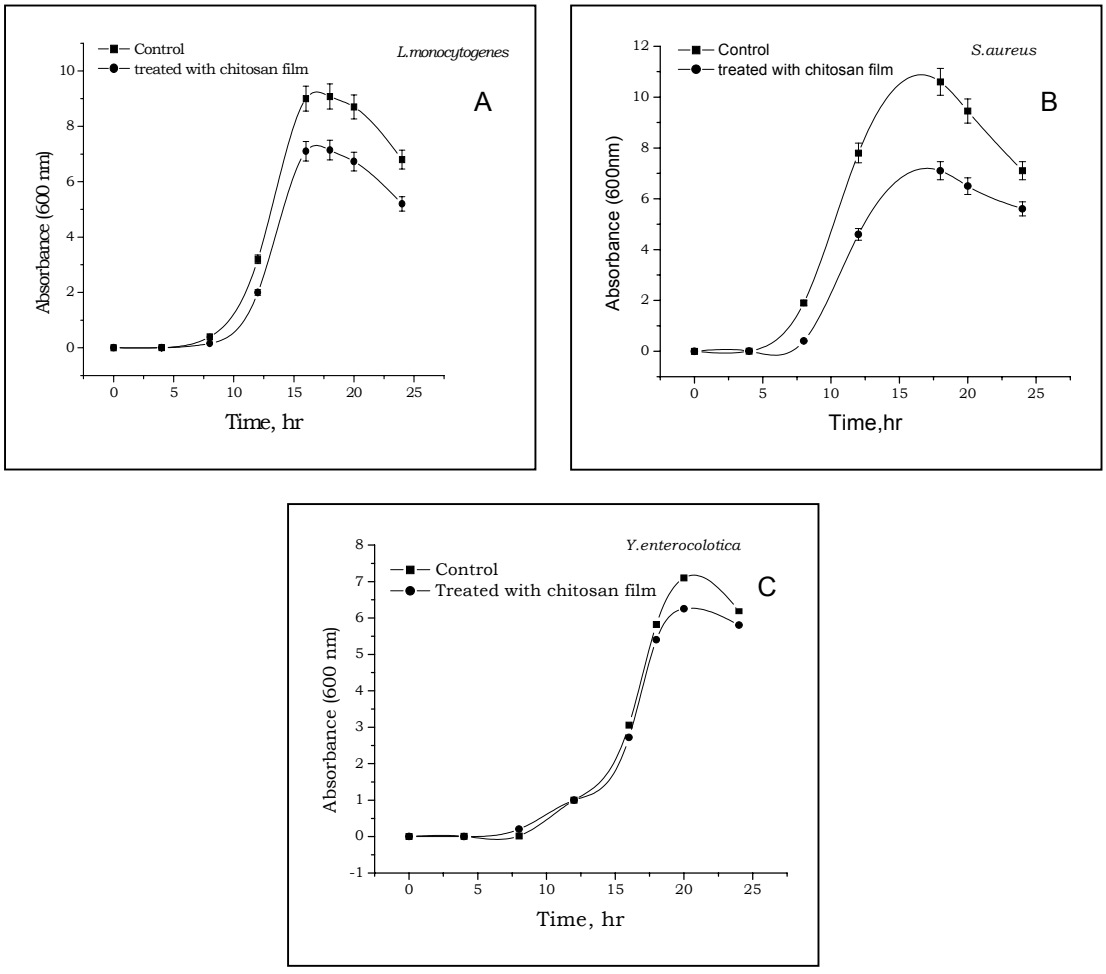

Fig. 5.1. Growth pattern of A). *L. monocytogenes* B). *S. aureus* C). *Y. enterocolitica*

(2002) showed reduction in the cell population only in the exponential phase of *L. monocytogenes* in the presence of 10%(v/v) chitosan solution. When compared to specific growth at the exponential phase, it was 0.16 compared to 0.26 in control. Similar observations made with *S. aureus* supports our data, wherein OD 600 at the experimental phase was 0.44 in the chitosan inoculated medium compared to 0.56 in the control tube, which drops to 0.39 at stationary phase of growth. In *Y. enterocolitica* the inhibition was observed right from the initial stage of the growth curve.

Cell density and inhibitory activity of chitosan

The medium supplemented with chitosan film showed good inhibition at lower cell concentrations for all the bacterial cultures selected. In order to detect the minimum inhibitory concentration of chitosan, the cell dilutions 3 $\log_{10}$, 4 $\log_{10}$ and 5 $\log_{10}$ cfu/ml were chosen. The strain of *L. monocytogenes* with varying concentration of chitosan film showed consistent pattern at all the three cell dilutions. It was observed, that irrespective of the initial concentration, the final concentration reached a maximum of 9.9 $\log_{10}$ cfu/ml in the control tubes by 48 hr, which gradually decreased to 9.2 log10 by 72 hr. Nevertheless, there was a gradual decrease in cell concentration with increasing chitosan concentration. It is evident from Fig 5.2A, that with enhanced incubation period upto 72 hr there was a notable decrease in cell number against higher increased cell dilution. Although the observations at 48 hr contradictly enhanced the cell growth, it could be because of bacteriostatic activity of chitosan. The earlier work of coma *et al.*(2002), where chitosan and acetic acid were added at log phase of growth curve of *L. monocytogenes,* and observed acetic acid has no effect on growth, while chitosan inhibit the growth of the microorganisms. Earlier work of Wang (1992) and Coma *et al.* (2002) on chitosan did not report a complete inhibition of the antilisterial property even after using chitosan in its pure form. Our results conclusively demonstrate the antibacterial effect of

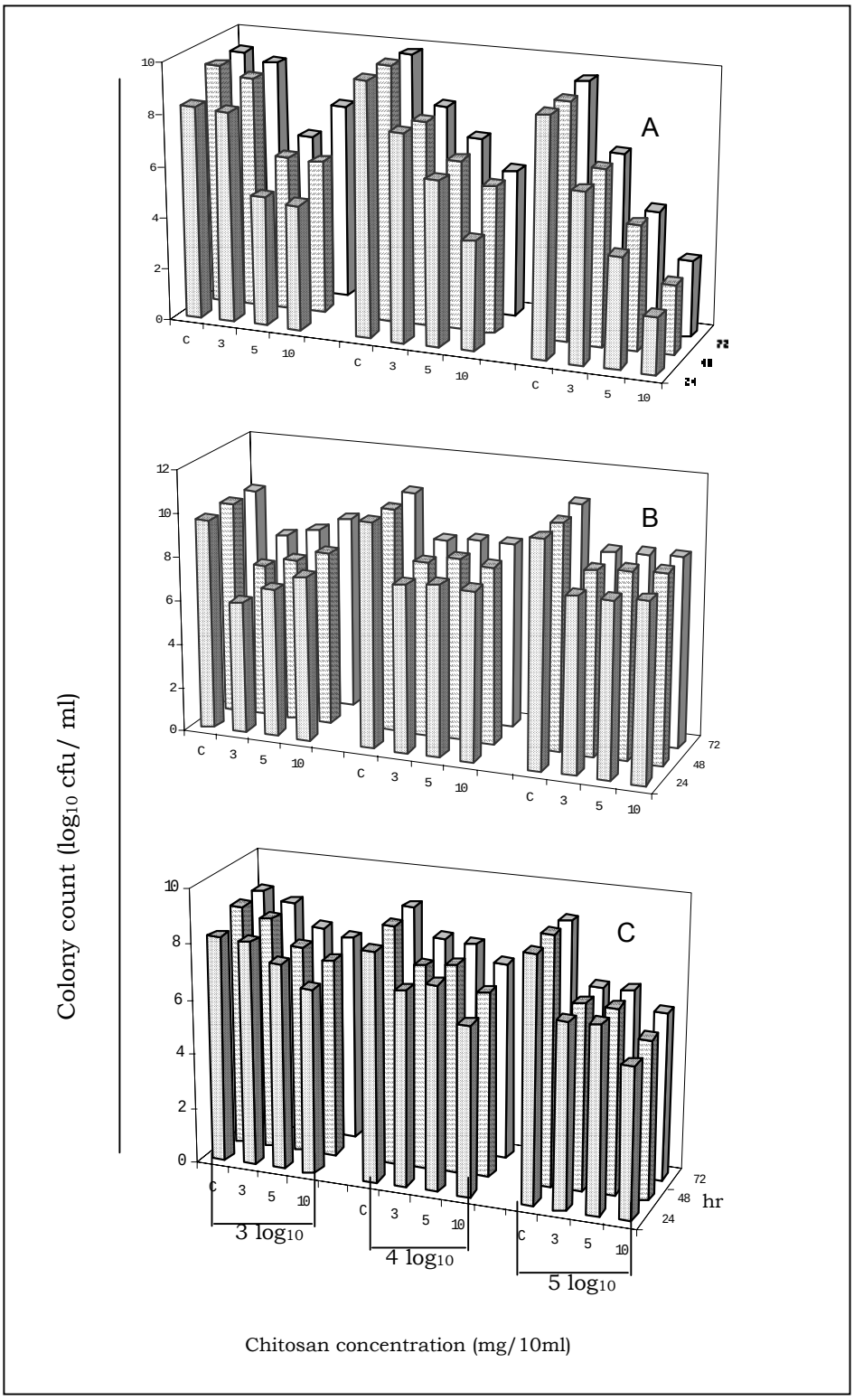

Fig. 5.2. Inhibition studies on A). *L.monocytogene*s. B). *S.aureus*. C). *Y.enterocolitica*

chitosan film in broth system, which calls for future applications in packaging films.

In the case of *S. aureus*, it was observed that during 24 hr of incubation, the cell count was less at all concentrations of chitosan compared to control (Fig 5.3b). Maximum inhibition was observed in tubes with initial inoculum level of 3 $\log_{10}$ cfu/ml. With increase in incubation time, more growth was observed in all experimental tubes. Chitosan concentration of 3 mg with initial inoculum level of 3 $\log_{10}$ cfu/ml could inhibit more efficiently compared to 5 and 10 mg concentrations. But in all cases reduced growth was observed in the presence of chitosan as against the growth in control tubes. Allan *et al.* (1984) reported that at 0.1% chitosan concentration, *S. aureus* inhibition was negligible or very less, whereas No *et al.* (2002) reported that the inhibition was dependent on the molecular weight, degree of polymerization, etc., of chitosan. SEM studies indicated the possible lysis of *S. aureus* upon exposure to chitosan, forming a characteristic pore with cup-like structure on the surface of films (Fig. 5.3b).

Fig. 5. 3. SEM of chitosan treated A). *L. monocytogene*s, B). *S. aureus,* C). Y. *enterocolitica*

As evidenced by the growth curve, *Y. enterocolitica* inoculated at an initial concentration of 3 $\log_{10}$ cfu/ml reached a maximum of 8 $\log_{10}$ cfu/ml by 24 hr, which on further incubation continued to grow till 9 $\log_{10}$ cfu/ml and later stabilized at 72 hr. Chitosan film showed the inhibitory effect on *Y.*

enterocolitica at both logarithmic and stationary phases of growth. At higher cell concentrations, its effect was less. At 3 mg, the cell mass decreased from 8 $\log_{10}$ in 24 hr to 5 $\log_{10}$ in 72 hr, whereas with 10 mg of chitosan, there was a drop in the growth from 9 $\log_{10}$ to 5 $\log_{10}$ cfu/ml. The antibacterial action of chitosan on Gram-negative bacteria may be due to its ability to bind and disrupt the permeability barrier of the outer membrane (Fig. 5.3c). The positive charge of amino group at C-2 below its pKa (pH 6.3) creates a polycationic character in chitosan structure, which can be expected to interact with the predominant anionic component of Gram- negative bacteria (Nikaido, 1996).

The antimicrobial property of chitosan is due to the polycationic nature of chitosan and its derivatives, which allow interaction and formation of polyelectrolyte complex with acidic polymers produced at the bacterial cell surface, such as lipopolysaccharide-teichoic and teichurnoic or capsular polysaccharide (Muzzarelli *et al.*, 1990).

In order to understand the mechanism involved in the repression of growth by chitosan film and to prove whether the effect is bacteriostatic or bactericidal, SEM studies were undertaken. As shown in Fig 5.3A, it was clearly seen that in the presence of chitosan film, *L. monocytogenes* sticks to surface of the film forming a clump (bacteriostatic) and inhibiting its further multiplication.

Antifungal property of chitosan film

Rhizopus sp. was observed to grow, whereas complete inhibition of *Fusarium* sp. was observed in the presence of chitosan films (Fig 5.4), and *Penicillium* species showed a partial inhibitory effect in the presence of chitosan film. The results indicated variations in the inhibition pattern among different fungal species.

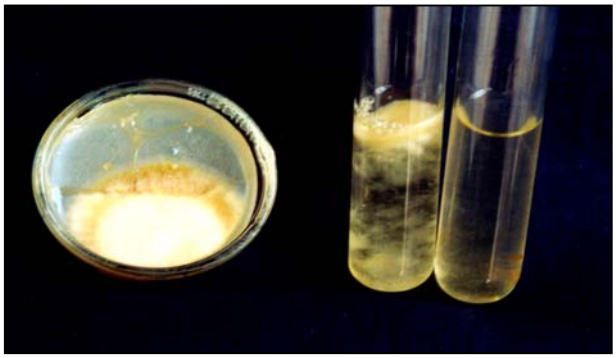

Fig. 5.4. Growth pattern of *Fusarium.* Sp.in solid and liquid media

The data showed a similar trend in liquid medium. Chitosan film had no effect in inhibiting *Rhizopus* species. *Fusarium* species did not grow in presence of chitosan film at lower or higher concentration levels, whereas in control flask, the dry weight of the species was 0.109 g (Fig. 5.5). Chitosan film showed a partial inhibitory effect of *Penicillium* sp. The fungal mat size was reduced in the presence of chitosan film (Fig. 5.5). The dry weight of fungal cell mass at 6 $\log_{10}$ dilution was 0.180 g in control, whereas in chitosan-inoculated flask, it was 0.081 and 0.108 in 100 and 20 mg, levels

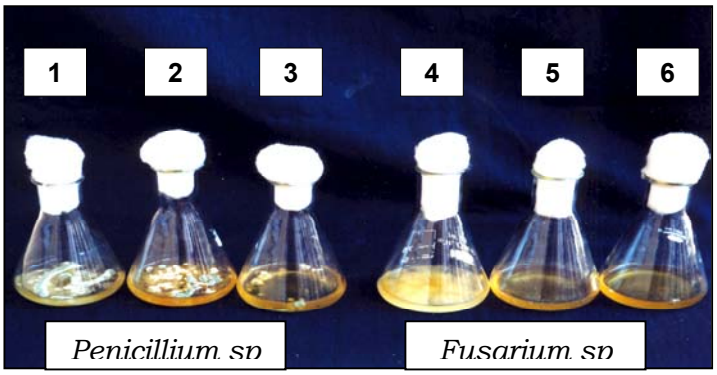

Fig. 5.5. Antifungal property of chitosan film against A.) *Pencillium sp.* B) *Fusarium sp.* at 1) control (1-4), 2) 20 mg/20 ml [2-5], 3) 100 mg/20 ml [3-6]

respectively. At lower concentration, growth was 0.163 in control and 0.087 and 0.041 mg in chitosan flask. The results show that with increase in

chitosan concentration the growth of fungi was decreased. Rodriguez *et al.* (2003) observed antifungal effect of chitosan on *Penicillium* in pizza, and observed 0.079 g of chitosan /100 g of pizza was effective in the control of the organism and the results were comparable to other preservatives used.

Conclusions

In comparison with the earlier data, our results conclusively demonstrated the antimicrobial property of chitosan film even at very low concentrations. Although there was lesser diffusion of chitosan on the agar surface, there was no growth, which makes it potentially useful in food preservation. SEM studies revealed the effectiveness of chitosan film as antimicrobial agent.

1. Polyester base material gave the best quality of chitosan films.

2. Infrared drying was the fastest of all the methods tried and no significant differences in film characteristics were observed between the various methods.

3. The WVTR of films increase with increase in RH, which is useful in the storage of fruits and vegetables.

4. The designed and fabricated wet casting unit produced chitosan films continuously. Further work, however is needed to optimize the various operating conditions and gadgets.

5. Chitosan-polyols blend films showed decrease in mechanical properties but improvement in barrier properties.

6. FT-IR showed major changes in polymer hydrogen bonding.

7. DSC results show a distinct endotherm peak at around 260 ˚C.

8. Fatty acid blend chitosan film did not show any significant change in the properties.

9. Addition of PVA significantly affected the barrier properties of the film.

10. Sorption studies showed GAB model to fit very well in all the modified films.

11. Chitosan film was very effective in extending the shelflife of mango at room temperature. The carotenoid level, total and reducing sugar levels were maintained for longer periods of storage. This result was supported by acceptable sensory analysis results.

12. The storage of tomatoes and bell pepper showed higher retention of colour. A uniform colour development was observed in chitosan packed vegetables.

13. Chitosan coated butter paper packed *peda* showed better quality characteristics.

14. Chitosan film and chitosan coated butter paper were not effective in extending the shelflife of bakery products, but incorporation of chitosan (at 0.1-0.2%) into the dough provided considerable extension of shelflife of bakery products.

15. Chitosan film inhibited the growth of certain types of microorganisms. The nature of inhibition was dependent on the type of microorganism.

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Research publications

a). Published

1. **Srinivasa, P.C**., Revathy Baskran, Ramesh M.N., Harish Prashanth, K.V., and Tharanathan, R.N (2002) Storage studies of mango packed using biodegradable chitosan films. ***European Food Research and Technology***. 215(6), 504-508.

2. **Srinivasa, P.C**., Kumar K.R., Ramesh M.N. and Tharanathan, R.N. (2003). Mechanical properties and sorption studies of chitosan and polyvinyl alcohol blends, ***Carbohydrate Polymers***, 54 (4), 431-438.

3. **Srinivasa, P.C**., Kumar K.R., Ramesh M.N. and Tharanathan, R.N (2002). "Drying characteristics of chitosan films" ***in Advances in Chitin Science***. Proceedings of 5th Asia pacific Chitin-Chitosan symposium and Exhibition, Bangkok, THAILAND, pp. 226-230.

4. **Srinivasa, P.C**., Ramesh M.N. Kumar K.R. and Tharanathan, R.N. (2004). Drying kinetics and properties of chitosan films prepared under different drying conditions, ***Journal of Food Engineering,*** 63 (1), 79-85.

5. **Srinivasa, P.C** ., Susheelamma N.S., Ravi R., and Tharanathan, R.N (2004) Effect of packaging films on quality of mango during storage**.** ***Journal of the Science of Food & Agriculture,*** 84,818-824..

b). Communicated.

1. **Srinivasa, P.C**., Padmapriya, B.P., Rati, E.R., Varadaraj, M.C., Tharanathan, R.N. Antimicrobial effect of chitosan-based packaging film on foodborne pathogens and spoilage microorganisms. (***European Food Research and Technology***)

2. **Srinivasa, P.C**., Ramesh M.N. and Tharanathan, R.N. Modeling sorption isotherm of modified chitosan film with plasticizers and fatty acids. (***Journal of Food Engineering***)

3. **Srinivasa P.C**., Harish Prashanth K.V., Susheelamma N.S., Ravi R., and Tharanathan R.N. Storage studies on tomato and bell pepper in chitosan based films in comparison to LDPE films. (***European Food Research and Technology***).

c). Under preparation

1. **Srinivasa, P.C**., Susheelamma N.S., Ravi R., and Tharanathan, R.N Storage studies of mango using eco-friendly films under different storage conditions.

2. **Srinivasa, P.C.** and Tharanathan, R.N. Application of eco-friendly chitosan films on food products.

3. **Srinivasa, P.C**., Kumar, K. R. Harish Prashanth, K.V. and Tharanathan, R.N. Modification of eco-friendly films by adding polyols, fatty acid and synthetic water soluble polymer.

4. **Srinivasa, P.C**., Susheelamma N.S., Ravi R., and Tharanathan, R.N Storage studies of vegetables using eco-friendly chitosan film.

Patents

1. Tharanathan, R.N., **Srinivasa, P.C.,** and M.N. Ramesh. A process for production of biodegradable films from polysaccharides. 85/DEL/2002

2. **Srinivasa, P.C**., M.N. Ramesh., Susheelamma N.S., and Tharanathan, R.N. A novel packaging for extending shelf life of fruits like mango and vegetables, 88/DEL/2003

3. **Srinivasa, P.C**., Susheelamma N.S., Rati Rao, E., M.N. Ramesh., and Tharanathan, R.N. A packaging process for extension of shelf life of dairy products (468/DEL/2004)