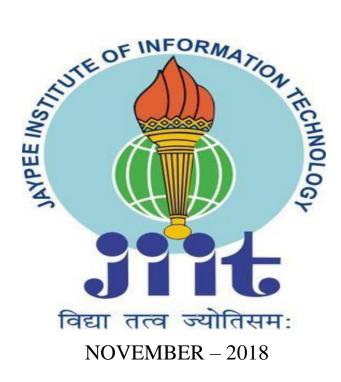
EDIBLE BILAYER FILMS AND COATINGS UNDER GRAS LIMIT FOR FOOD PACKAGING

ENROLMENT NO-16101026

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Submitted in partial fulfilment of the Degree of Bachelor of Technology

DEPARTMENT OF BIOTECHNOLOGY

JAYPEE INSTITUTE OF INFORMATION TECHNOLOGY,

NOIDA

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CERTIFICATE

This is to certify that the work titled "EDIBLE BILAYER FILMS AND COATINGS UNDER GRAS LIMIT FOR FOOD PACKAGING" submitted by "JASVEEN BHASIN" in partial fulfilment for the award of degree of B. TECH IN BIOTECHNOLOGY of Jaypee Institute of Information Technology, Noida has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor:

Name of Supervisor: Ms Manisha Singh

Designation: Associate professor

Date: -November-2018

ACKNOWLEDGEMENT

This project consumed huge amount of work, research and dedication. However, it would not

have been possible without the kind support and help of few individuals. I consider it as a

privilege to express through the pages of this project report, a few words of gratitude and

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I would like to take this opportunity to express my heartfelt gratitude to my Mentor Ms Manisha

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project.

I would also thank my team members for their willingness to help and co-operation throughout

my entire project work.

Signature of the student:

Name of Student: - Jasveen Bhasin

Date: - NOVEMBER-2018

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SUMMARY

This study is mainly focused on the use of the edible bilayer films and coatings under GRAS limit for food packaging, replacing the conventional nonedible polyethylene- and polypropylene-based packaging materials to prevent the environmental problems thus increasing ecological consciousness. The biodegradable edible films generally made of biopolymers such as polysaccharides, proteins, lipids and their blends serve as potential packing material as they have the potential to enhance food quality, safety, shelf life and mechanical strength also acting as the barriers against mass diffusion of gases, moisture, volatiles. The effect of polysaccharide based coating on the fruit strawberry was studied during cold storage where alginate, chitosan and pullulan were applied to the postharvest strawberry and their effect on firmness, decay rate, respiration rate, ascorbic acid content, titrable acidity, CAT and APX activity was observed where chitosan was found to have the most positive effect on the fruit quality, presenting the highest relative activities of antioxidant enzymes. The influence of lipids used in edible films, mainly for their efficiency as water- vapour barriers was also evaluated. Edible films act as carriers of various additives, such as antioxidants, antimicrobial, nutraceuticals, flavouring, colouring and crosslinking agents and thus they have great potential to deliver functional compounds to the food. The study involves the technique of Encapsulation of such bioactive compounds. The combined effect of plasticizers and surfactants on the physical properties of edible films is also discussed. The study concludes with the methods used to evaluate the edible films based on their mechanical and barrier properties.

Signature of Student	Signature of Supervisor
Name:	Name:
Date	Date

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INTRODUCTION

The food-packaging system is to provide protection to the food products from the factors, such as oxygen, heat, enzymes, moisture, micro- and microorganisms thus acting as barriers, preventing unpleasant colour and odour. They should also be successful in preventing the loss of aroma. Today in the global market there is increasing demand for stable products thus increasing the demand for food-packaging materials that can retain the natural properties of the food products, their quality is maintained for a longer period of time. Thus there arises the need for edible films and coatings. [1] Edible films are any type of thin material which is used for enrobing that is wrapping or coating food material and drugs to extend the shelf life of product that may be consumed with food or removed before consumption. The term "edible film" has two main considerations. "Edible" tells that the film can be directly consumed with the food product and the film also contains all properties of safe- food ingredients that are approved by Food and Drug Administration (FDA) and got a status of GRAS (Generally Recognized as Safe). "Films" means that wrapping material that can protect the inner food product from the outer environment and also possess all packaging properties like limiting gas and water vapour transportation, providing mechanical and physical strength. [2]

Edible films and coatings are produced mainly from biopolymers which are polysaccharides like alginate, chiton; proteins which include gelatin, casein. These materials impart crispness, compactness, hardness, viscosity, thickening quality, and adhesiveness to the edible films. These films have good gas permeability, prevents the anaerobic conditions, good mechanical properties but because of their hydrophilic nature, they are poor barriers of moisture content. Thus lipids which may be wax and glycerides are used which have poor mechanical strength but are good barriers to water vapour. Different methods used to apply these edible films on food products include dipping, spraying, brushing, and panning in which a thin coating forming the semipermeable membrane on the surface of food products is produced. [1]

Factors like storage conditions, addition of food additives affect the quality of the edible films and coatings. Thus to enhance the properties of films active ingredients like antimicrobial, antibrowning, antimicrobial agents, flavouring agents, nutraceuticals, texture enhancers, colorants, flavours are incorporated. [1] Mechanical and barrier properties of films by different methods which are explained in the report.

ADVANTAGES OF USING EDIBLE FILMS OVER SYNTHETIC FILMS

- 1. The edible films and coatings are produced from ingredients that are food grade in order to maintain their edibility and can be consumed with the food product directly.
- 2. Using the edible films which have the ability of holding the juices in the fresh fish cut, meat, or poultry, and preventing the dripping, which cannot be achieved by using synthetic coatings, reduction of moisture loss and hence retention of flavour, appearance, texture, weight can be maintained.
- 3. Edible films act as a barrier for the gas exchange between food product and the surrounding environment, reducing the rate of respiration, oxidation and decay. They also act as a barrier for the transfer of volatile compounds preventing the loss of odour and flavour of the food.
- 4. Edible films provide mechanical strength and protect the food from the physical damage caused by the external pressure and vibration.
- 5. Edible films act as the carrier of different active ingredients and food additives such as antimicrobial, antibrowning, antioxidant agents, flavours and nutraceuticals.
- 6. The browning and rancidity which is caused by the myoglobin oxidation in the meat can be prevented by using the edible packaging system. [1]

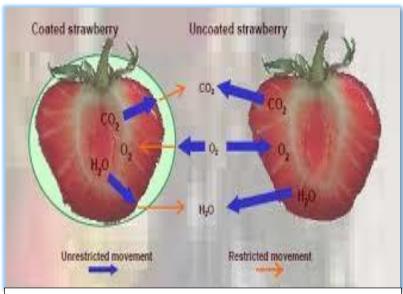
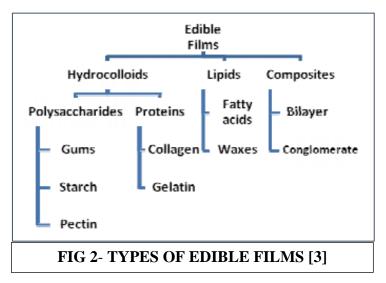


FIG 1- Movements restricted by edible films and coatings

[3]

MATERIALS USED IN EDIBLE FILMS AND COATINGS

A large number of materials is used to produce edible films and coatings, but the most of them can be included in one of these three categories: Polysaccharides, Proteins and Lipids.



(1) Polysaccharide films- They are made from chitosan, alginate, starch, cellulose ethers, carrageenan, or pectin and are efficient to impart compactness, hardness, viscosity, thickness, crispness, adhesiveness and gel forming ability to a number of edible films. These films are made of the polymer chains thus exhibit excellent gas permeability properties, mechanical and structural properties and help in preventing oxidative rancidity, and surface browning, increasing the shelf life of the food product but their hydrophilic nature makes them poor barriers for water vapour. [3]

Coating	Food Product
Chitosan	Table Grapes
Corn Starch	Cucumber
Chitosan with cinnamon oil	China jujube
Alginate-Chitosan	Fresh cut melon
Carboxymethyl cellulose	Cucumber

Table 1- Polysaccharide- based edible coatings applied on different food [3]

(2) **Protein Films-** Proteins are polymers that contain more than 100 amino acid residues. They must be denaturated by alkali, acid, heat to form extended structures that are required for formation of the film. Compared with synthetic and lipid films, these films show poor water resistance but are still superior as they possess the ability to form films with greater mechanical and barrier properties. Physical and chemical properties of these films are influenced by electrostatic charge, amino acid composition, secondary, tertiary and quaternary structure changes due to heat, acid, alkali, pressure, heat, mechanical damage, enzyme action. [4]

Coating	Food Product
Whey Protein	Fresh Mutton
Gelatin	Acerola fruits
Corn Zein	Intermediate moisture apricot
Gelatin	Strawbwerries
Whey protein- gellan gum	Apples

Table 2- Protein- based edible coatings applied on different food [3]

(3) **Lipid Films**- The efficiency of lipid materials in edible films and coatings depends on the nature of the lipid, hydrophobicity, its structure, chemical arrangement, physical state, lipid interactions with other components of the film. Lipids are usually combined with other film-forming materials, such as proteins or polysaccharides because of low mechanical strength in order to increase the resistance to water penetration. [4]

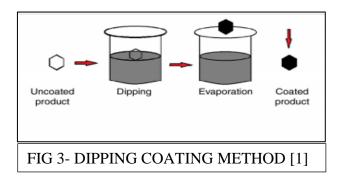
Coating	Food Product
Whey Protein	Fresh Mutton
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Table 3- Polysaccharide- based edible coatings applied on different food [3]

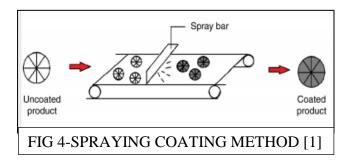
METHODS TO APPLY EDIBLE FILMS ON FOOD PRODUCTS

There are different methods used to apply edible films on food products like dipping, spraying, brushing, panning, fluidized bed coating, dripping.

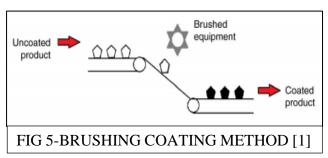
(1) **DIPPING-** In this method coating is made by dipping in a coating solution with properties such as viscosity, surface tension, density and also food withdrawal speed from the coating solution. [3]



(2) **SPRAYING-** This method is used when the solution for forming coating is not very viscous. The control of the final drop size and thus the quality of the coating depends on flow rate of air and liquid, nozzle and spray gun temperature, humidity of incoming air and also of the polymer solution. [3]



(3) **BRUSHING**- It is used for the applying film solution to fresh beans and strawberries preventing water loss. [3]



EFFECTS OF POLYSACCHARIDE- BASED COATINGS ON QUALITY AND ANTIOXIDANT ENZYME SYSTEM OF STRAWBERRY DURING COLD STORAGE

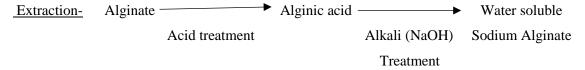
Strawberry is a nutritious fruit but is highly perishable fruit. Thus it was coated with three polysaccharide-based edible coatings alginate, chitosan, and pullulan while postharvest during cold storage (4° C).

In this study the effect of three polysaccharide coatings on the quality of strawberry was investigated.

The changes in the properties of the uncoated (control) and the coated were recorded and the difference in properties were compared between the coated and uncoated fruit and the fruit with three different coatings. [5]

Polysaccharides used-

(1) ALGINATE- A polysaccharide derived from marine brown algae (Phaeophyceae) and gellan or secreted by bacterium Sphingomonas elodea. It found in the cell walls of brown algae of family Phaeophyceae. It is linear, unbranched and anionic.



They are block polymers having beta (1→4) linked D- mannuronic acid and alpha (1→4) linked L-gulluronic acid.

- (2) CHITOSAN- A high molecular weight polysaccharide obtained by deacetylation of chitin by Sodium hydroxide solution at high temperature or by enzymatic deacetylation is a byproduct of seafood industry. It is found in the exoskeleton of arthropods, crabs, lobsters, crustaceans and mollusks. It is linear, unbranched cation with beta (1 → 4) glycosidic linkage.
- (3) **POLLULAN-** It is a polysaccharide consisting of maltotriose units and is produced by the fungus Aureobasidium Pollulans in the cell wall. It is edible and tasteless. [5]

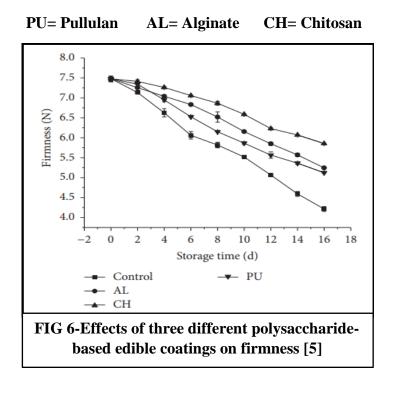
RESULT AND DISCUSSION-

(1) Effect on Firmness- It was seen that in the fruits coated with the polysaccharide based coating the firmness decreased slowly with much slower rate when compared to the uncoated strawberry (control).

This was due to the reduction in water loss by slowing the metabolic processes like transpiration and respiration when the fruit was coated with polysaccharide based coating. Thus the weight loss was less as the stomata and guard cells were blocked, the active metabolic processes were slowed down.

Also firmness depends on the softness. As softness increases firmness decreases. Softening increases with progression in ripening due to depolymerisation of pectin substances where softening enzymes including polygacturonase and pectin esterase alter the cell wall causing softening. Edible coatings inhibited pectin enzyme by slowing metabolic processes. Also the coatings developed resistance against composition changes in cell wall and moisture loss. It was also observed that that there was less water and nutrient loss in fruits coated than uncoated strawberries

All these reasons were responsible for high firmness in the coated strawberries with maximum firmness in strawberries coated with Chitosan which is shown in the graph below.



(2) Effect on Decay Rate- Decay rate is inversely proportional to the firmness. As firmness was high in the strawberries coated with polysaccharide coatings and was maximum in the strawberries coated with chitosan. It was seen that the decay rate was maximum in the uncoated strawberries while minimum in the strawberries coated with chitosan as there was reduction in water and nutrient loss with inhibited and slow rate of softening in the coated strawberries which is shown in the graph below.

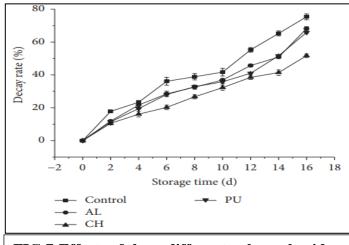


FIG 7-Effects of three different polysaccharidebased edible coatings on decay rate [5]

(3) Effect on respiration rate- Coating on strawberry prevent oxidative reactions strengthening the antioxidant system of the fruit. It was seen that the rate of respiration was maximum in uncoated strawberries that had to bear the maximum oxidation of nutrients and acids and thus there was maximum oxidative damage while chitosan had minimum respiration rate as it acted as gas barrier.

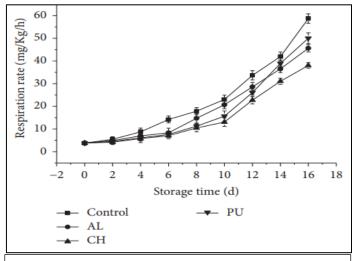


FIG 8-Effects of three different polysaccharidebased edible coatings on respiration rate [5]

(4) Effect on Ascorbic Acid content and Titrable Acidity- Due to increased respiration rate in the uncoated strawberries there was seen increased oxidation of acids into sugars thus decreasing the ascorbic acid content as compared to the strawberries coated with different polysaccharide- based coatings in which there was low rate of respiration thus less oxidation of organic acids in sugars and thus high titrable acidity that is measure of content of acid. In the strawberries coated with chitosan there was maximum ascorbic acid content and titrable acidity as the rate of oxidation of acid was slow and thus acidity was maximum.

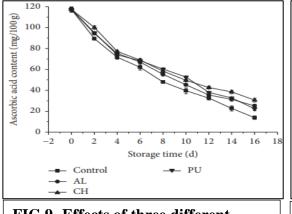


FIG 9- Effects of three different polysaccharide- based edible coatings on ascorbic acid content [5]

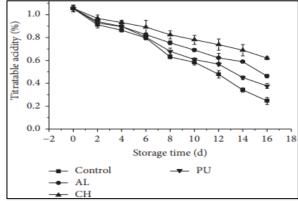


FIG 10- Effects of three different polysaccharide- based edible coatings on Titrable acidity [5]

(5) Effect on CAT (Catalase) and APX (Ascorbate Peroxidase) Activity- These antioxidant enzymes reduce overproduction of ROS (Reactive Oxygen Species), preventing disturbed bodily homeostasis, delaying oxidative damage as ROS are oxygen centred molecules with unpaired valence shell electron which are unstable, highly reactive with proteins, lipids in cell. The activity of these enzymes decreased but was comparatively high in chitosan.

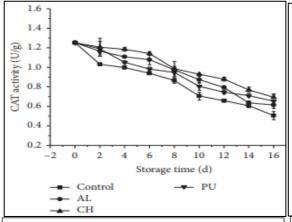


FIG 11- Effects of three different polysaccharide- based edible coatings on CAT Activity. [5]

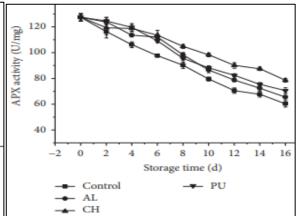


FIG 12- Effects of three different polysaccharide- based edible coatings on APX Activity. [5]

ENHANCEMENT OF THE PROPERTIES OF EDIBLE FILMS USING FOOD ADDITIVES

Food additives are the organic substances that are added to food product usually in small quantities during production or processing generally to improve the organoleptic quality of the food that is the colour, taste, appearance, texture, odour and flavour.

On incorporation of the food additives, edible films act as the carrier of these special ingredients i.e. the flavouring and active substances and have a great potential to deliver these bioactive compounds to the food improving their quality and functionality. [6]

Food additives can be incorporated as antioxidants, antimicrobial agents, antibrowning agents, nutraceuticals, plasticizers, surfactants, flavouring and colouring agents.

Food additives can either be used directly or indirectly. Direct additives are the ones that are added intentionally to foods for a specific purpose while indirect additives are the ones to which the food is exposed during the processes of processing, packaging, or storing. [6]

Through the process of migration, the bioactive compounds included in the coated polymer matrix can be transferred to the food product or headspace where they pursue their protective action.

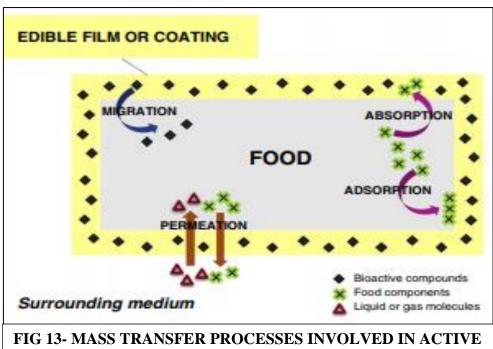


FIG 13- MASS TRANSFER PROCESSES INVOLVED IN ACTIVE FILMS AND COATINGS [7]

FOOD ADDITIVES USED IN EDIBLE FILMS AND COATINGS

There are variety of bioactive ingredients that can enhance the organoleptic, physical, nutritional properties of the films when added with the polymer. Few are-

- PLASTICIZERS- They are small molecules like glycerol, polyethylene and
 polypropylene glycol that can modify mechanical properties of films and coatings, are
 able to provide transparent and intact films. They have lower values of oxygen
 transmission rate and water vapour permeability.
- 2. **SURFACTANTS-** They are substances that absorb to interfaces or surfaces causing decrease in surface tension. In order to improve wettability and adhesion of the film surfactants are incorporated into the film formulation.
- 3. **ANTIOXIDANTS** They are the substances which inhibit oxidation that counteract the detoriation of the food material that is stored or preserved.
- 4. **ANTIMICROBIALS** They are the substances that inhibit the growth or destroy the microorganisms specially that are pathogenic and can cause detoriation of the food material stored.
- 5. **ANTI- BROWNING AGENTS-** They are the additives that function to prevent or control the enzymatic browning in foods such as fruit and meat.

Plasticizers	Polyols	Glycerol, propylene glycol, polypropylene glycol, sorbitol, polyethylene glycol, corn syrup	
	Others	Sucrose and water	
Additives	Flavors	Oil based flavors, Citrus, Mints, Volatile oils	
	Colors	Pigments	
	Antimicrobials	Organic acids (acetic, benzoic, lactic, propionic, sorbic); Fatty acid esters (glyceryl monolaurate) Antimicrobials Polypeptides (lysozyme, peroxidase, lactoferrin); nitrites and sulfites, chitosan, bacteriocins (nisin, pediocin), parabens, liquid smoke, sodium chloride.	
	Antioxidants	Ascorbic acid, 4-hexylresorcinol, amino acids (cysteine and glutathione), citric acid.	
	Nutrients	Vitamin E, calcium, zinc, aluminum	
	Emulsifiers	Lecithins, mono- and diglycerides, mono- and diglyceride esters, Fatty sucrose esters, fatty alcohols, fatty acids	
	Lipid emulsions	Edible waxes, fatty acids	
	Probiotic organisms	Bifidobacterium (Bifidobacterium lactis Bb-12)	
	Plant essential oils	Cinnamon, oregano, lemongrass, savory, sweet inula, vanilin, clove, citronella, thyme	

TABLE 4- FOOD ADDITIVES IN EDIBLE FILMS AND COATINGS

[8]

ENCAPSULATION OF FLAVOURS, NEUTRACEUTICALS, ANTIMICROBIALS

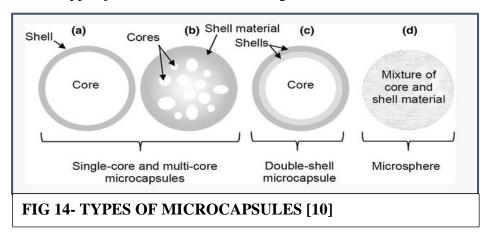
Encapsulation is the technique of coating to protect and preserve minute particles of ingredients (e.g. flavours, acidulants, antimicrobial, fats) as well as the whole ingredients (nuts, raisins and confectionery products). This is accomplished by microencapsulation and macro- coating techniques, respectively.

REASONS FOR ENCAPSULATION-

- 1. As the bioactive compounds are volatile they have to be retained in the food product during storage.
- To protect them from undesirable interactions with the food. E.g. The case of flavour/ food interaction in chewing gum. Adding liquid flavour to chewing gum results in substantial losses of flavour of the gum base and the flavour is not recovered during chewing.
- 3. To minimise flavour/flavour interactions. If the flavour contains reactive constituents (e.g. aldehydes and amines which would form Schiff's bases). Each could then be encapsulated separately and blended into the finished dry food product.
- 4. To guard against either light-induced reactions or oxidation.
- 5. To effect the controlled release of the flavour. [9]

CAPSULE MATRICES- Encapsulation of biomolecules can be achieved by using two main methods-

- 1. The first is making capsules in which the compound included as a core is entrapped in a polymeric matrix.
- 2. The second method is developing films or coatings in which biomolecules are directly included and trapped just as a matrix but on a larger scale.



THE EFFECT OF CHITOSAN COATING INCORPORATED WITH ETHANOLIC EXTRACT OF PROPOLIS ON THE QUALITY OF REFRIGERATED CHICKEN FILLET

In this study evaluation was done of the coating of chitosan (2%) containing ethanolic extract of propolis (1% and 2%) on microbiological (Staphylococcus aureus, psychotropic, lactic acid bacteria, coliforms and mesophilic aerobic counts) properties of chicken fillet.

Propolis is a substance derived from plant resins, which is collected by bees and converted into wax- like condensation product and is used for infections caused by bacteria, viruses, fungus, and by single-celled organisms called protozoans due to its antimicrobial, anti- viral, antioxidant, anaesthetic property. Ethanol extract of Propolis improve the properties of chitosan edible coating in chicken fillet preservation. [11]

RESULT AND DISCUSSION-

1. **Effect on aerobic mesophilic bacteria count**- It was seen that after 12 days' storage of chicken fillet the minimum number of bacterial count was in chicken fillet coated with chitosan+ 2% propilis while it was more in fillet that was only coated with chitosan and maximum in uncoated chicken.

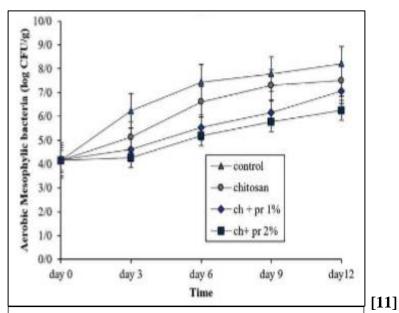


FIG 15- Total aerobic mesophilic bacteria count in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days' storage at 4 8C

2. Effect on Psychotrophic bacteria count –It was found that here again after 12 days' storage of chicken fillet minimum number of bacterial counts were found in fillet coated with chitosan+2% propolis. While the number of bacteria in uncoated fillet and fillet coated with chitosan was overlapping.

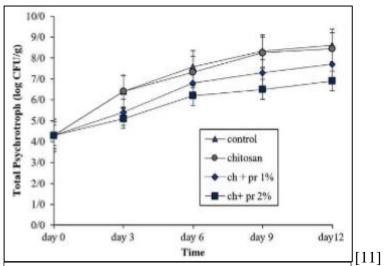


FIG 16- Psychotrophic bacteria count in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days' storage at 4 8C

3. Effect on bacteria S. aureus-

Treatment	Storage days				
Treatment	0	3	6	9	12
Control	0.00 ^a *	0.00 ^a	$1.61\pm0.02^{\mathrm{a}}$	$\textbf{1.53} \pm \textbf{0.01}^{a}$	$3.44\pm0.06^{\text{a}}$
Chitosan	0.00 ^a	0.00 ^a	$1.92\pm0.04^{\mathrm{a}}$	$2.54\pm0.04^{\mathrm{b}}$	$3.93\pm0.01^{\text{a}}$
Chitosan + 1% Propolis	0.00 ^a	0.00ª	0.00 ^b	0.00°	2.62 ± 0.01^{b}
Chitosan + 2% Propolis	0.00 ^a	0.00ª	0.00 ^b	0.00 ^c	1.30 ± 0.02^{c}

[11]

As seen, if the extract of propolis used alone (liquid form) like spraying it on filets, after a while due to its volatile nature the concentration of the product was reduced which resulted in less antimicrobial effects. Chitosan coating was effective in reducing number of gram negative bacteria. Studies proved that propolis affects gram positive bacteria more than gram —ve ones due to the outer membrane of gram negative bacteria that limits penetration and diffusion of hydrophobic compounds in lipo- polysaccharide that cover the bacteria.

COMBINED EFFECT OF PLASTICIZERS AND SURFACTANTS ON THE PHYSICAL PROPERTIES OF STARCH BASED EDIBLE FILMS

In this study films made of potato starch were developed in which glycerol was used as plasticizer and Tween 20, Span 80, and soy lecithin were used as surfactants in the formulation. Films were characterized with respect to mechanical properties and water vapour permeability (WVP). The wettability of the film solutions was quantified by measuring surface tension.

It was Incorporation of plasticizers that resulted in more flexible films and higher Water Vapour Permeability. It was seen that there were synergistic effects in between plasticizers and surfactants while higher level of surfactant concentration resulted in lower tensile strength, higher elongation, and higher WVP thus showing all the properties of films with large amount of plasticizers. [12]

RESULTS AND DISCUSSION-

1. Effect on Surface Tension: Three concentration of surfactant (0%, 0.5%, 5%) were taken with respect to starch content. Starch and glycerol are not tensio-active substances so they did not affected the surface tension significantly. Higher was the concentration of surfactant lower was surface tension. Results showed that Tween 20 was the surfactant with more uniform behaviour, was successful in lowering or diminishing the surface tension even at low concentration of 0.5% when compared with the other surfactants i.e. Span 80 and soy lecithin needed higher amounts to be effective.

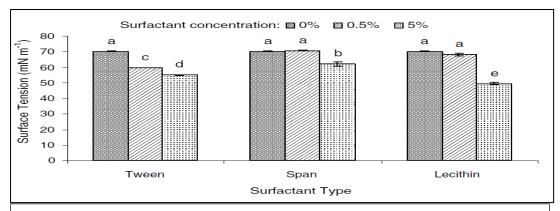
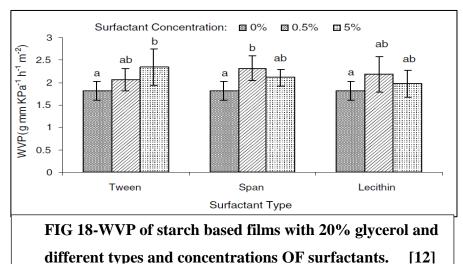


FIG 17-Surface tension changes of film formulations with 20% glycerol with different types and concentrations of surfactants. [12]

- 2. **Effect on Film characterization**: It was seen that on incorporation of glycerol, starch films were easy to handle and more flexible. It was found that the best concentration of glycerol with respect to starch is 20% (w/w). When this limit was made to exceed, phase separation was observed, which led to undesirable adhesiveness of the film. Also it was found that if only surfactant was added in absence of glycerol, it led to brittleness in the films formulation. [12]
- 3. Effect on Water vapour permeability (WVP): Glycerol is a small hydrophilic molecule, which when inserted adjacent polymeric chains decreases intermolecular attractions thus, increasing molecular mobility facilitating the migration of water. When the surfactant was added it was expected that WVP would decrease which was rightly observed in Soy lecithin and Span 80 but Tween 20 was exception as its higher concentration led to increase in WVP. This was due to high HLB (Hydrophilic-lipophilic Balance) ratio of glycerol making it hydrophilic.



4. **Effect on mechanical strength-** The expected result of glycerol interfering with starch packing, decreasing intermolecular attraction and thus increasing mobility was found. This involved an increase in elongation and a decrease in tensile strength. Films with glycerol in the presence of high level of surfactant behaved as films with larger amount of plasticizers (with lower tensile strength and higher elongation) telling about the synergistic behaviour between surfactants and plasticizers.

CONCLUSION

Today, in the food market the use of edible films and coatings is constantly increasing. Coatings are successful in helping and meeting many challenges both related to the storage and marketing of perishable food products. The performance and functionality of edible films and coatings are based on their barrier and mechanical properties, which in turn depend on the composition of the film, the process used in its formation and the method of application used to apply it the product. The properties of the film can be increased by adding food additives, which when encapsulated show better and regulated results.

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PLAGARISM CHECK

