

INTERDISCIPLINARY PROJECT REPORT
ON
**PREPARATION OF STARCH BASED EDIBLE FILM
INCORPORATED WITH CISSUS QUADRANGULARIS
FIBER**

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Engineering/Technology degree in Sathyabama Institute of Science and
Technology

by

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BONAFIDE CERTIFICATE

This is to certify that this Interdisciplinary Project Report is the bonafide work of **AMBRITHA C (39230001), ANITHA V (39230003), KEERTHANA S (39230022), SUBHASHINI R (39230046)** who carried out the Interdisciplinary Project entitled "**PREPARATION OF STARCH BASED EDIBLE FILM INCORPORATED WITH CISUS QUADRANGULARIS FIBER**" under our supervision from February 2022 to April 2022..

Year Coordinator

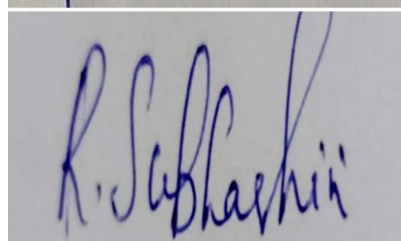
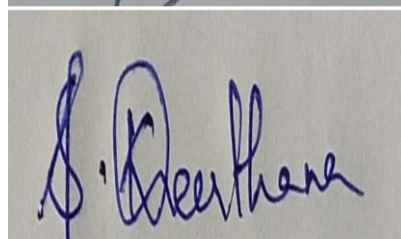
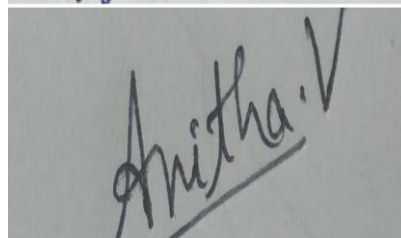
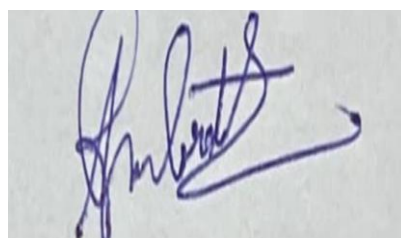
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DECLARATION

We hereby declare that the Interdisciplinary Report entitled “**PREPARATION OF STARCH BASED EDIBLE FILM INCORPORATED WITH CISUS QUADRANGULARIS FIBER**” done by us is submitted in partial fulfillment of the requirements for the Interdisciplinary Project.



DATE: 28/04/2022

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ABSTRACT

Cissus quadrangularis, is widely accepted natural medicine for bone setting in Indian Ayurveda. The GC-MS analysis of this plant stem extract also confirmed the presence of phyto chemicals such as octadrine, piperidinol, phytol in it. Edible films (EFs) at different concentration of corn starch, glycerol and *C. quadrangularis* stem extract using Box Behnken method under Response Surface methodology. Thickness of the prepared EFs varied between 79-136 μ m and the opacity of the prepared EFs in the range of 20.31-30.45%. Tensile strength (S) (0.98-5.49 MPa) of the EFs was decreased significantly with increase in glycerol content. Increase in plant extract concentration significantly decreased TS of the EF. Water vapor permeability (0.739×10^{-7} g/m. s. pa - 10.9×10^{-7} g/m. s. pa) of the EFs significantly decreased with increased starch, glycerol and plant extract concentrations. EFs with 15% plant extract showed antimicrobial effect on *E. coli* bacteria. Optimized EF formulation contains 3.33, 20 and 15 per cent starch, glycerol and *C. quadrangularis* stem extract.

Contents

1) BONAFIDE CERTIFICATE	i
2) DECLARATION	ii
3) ACKNOWLEDGEMENT	iii
4) ABSTRACT	iv
5) LIST OF FIGURES	vi
6) LIST OF TABLES	vii
7) LIST OF SYMBOLS AND ABBREVIATION	vii
8) INTRODUCTION:	1
9) LITERATURE SURVEY.....	2
(i) MATERIAL AND METHODS.....	2
(ii) EDIBLE FILM PROPERTIES.....	3
(iii) RESULTS AND DISSCUSSION.....	4
(iv) CONCLUSION.....	6
10) AIM AND SCOPE.....	6
11) MATERIALS AND EXPERIMENTAL METHOD.....	8
(i) METHODOLOGY.....	8
(ii) MATERIALS REQUIRED.....	17
12) RESULTS AND DISCUSSION.....	18
(i) RESULT.....	18
(ii) DISCUSSION.....	19
13) CONCLUSION.....	20
14) REFERENCE.....	21

LIST OF SYMBOLS AND ABBREVIATION

1) EF	Edible Film
2) WVP	Water Vapour Permeability
3) GCMS	Gas Chromatography-Mass spectrometry
4) DBM	Double Boiling Method
5) TGA	Thermogravimetric Analysis
6) FTIR	Fourier Transform Infrared spectroscopy
7) EDAX	Energy Dispersive x-ray Analysis

INTRODUCTION:

Edible films and coatings are thin layers that are coated on the surface of food products. Beyond those coming from the conventional ones, the main functions of this type of packaging are edibility and biodegradability. In addition, films are water soluble and dissolve by almost 90% in 24 hours. Although many bio-films including whey gluten, were found suitable for packaging, none of them ever tested so far add better outcomes than the existing bio-films and therefore bio-films are commercially not viable to date. Hence in this project work, a novel bio-film additive derived from naturally available herbal species known as *Cissus quadrangularis* has been proposed. One major advantage of using edible films and coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes.

Edible films protect the fresh fruits and vegetables, cut fruits and minimally processed fruits by maintaining its structural integrity towards integrated product. This make them better alternate to the synthetic polymer packaging materials (Chiumarelli and Hubinger 2012). Several naturally available polysaccharides, proteins and lipids are used as a source for edible film preparation. Among those, starch is a promising packaging material due to its tasteless, odorless, transparent, inherent and better film forming character (Choiet al 2016). Starch can be obtained from the several sources like potato, tuber, cassava and corn. Corn starch widely produced cheap source of polysaccharide available all over the world (Laycock and Halley 2014) and contains all essential amino acids and digestible proteins, except tryptophan (Duconseille et al 2015). Despite having numerous advantages as a film, but it does not have thermoplastic property. Hence, it cannot be used as a packaging material directly, without plasticizer (Dang and Yoksan 2015). Addition of plasticizer improves the flexibility but decreases the mechanical resistance (Azeredo et al 2012). Physical and mechanical barrier properties of the film also depend on the concentration of the plasticizer (Sahariet al 2012). Glycerol is the most promising plasticizer, due to its

better compatibility with starch. Now a days EFs functionality was increased by incorporating antimicrobial agents, antioxidants, functional ingredients such as probiotics, minerals and vitamins (Campos et al 2011). Some of the plants, animals, bacteria, algae and fungi are rich source of antimicrobial component. Among them plant antimicrobials have higher efficacy in food applications (Ulbin-Figlewicz et al 2013). In that aspect, (Devil bone) *Cissus quadrangularis* stem contains rich source of antioxidant and antimicrobial properties due to the presence of polyphenolic compounds. Saponins, flavonoids (Indran et al 2014). So far, no work has been conducted on the use of stem *Cissus quadrangularis* extract and its effect on the EF properties. Hence this study was undertaken to develop and characterize the EF at varying concentration of corn starch, glycerol and *Cissus Quadrangularis* stem extract.

CHAPTER-2

LITERATURE SURVEY

MATERIALS AND METHODS

Preparation of plant extract:

The *Cissus quadrangularis* plants were collected from the local market and nearby gardens. Plant extract was obtained by following the procedure prescribed by Hanumantharaju et al.

GCMS analysis of the *Cissus quadrangularis* stem extract:

GCMS analysis of plant extract samples were performed on GC-MS analyzer (Model: TRIPLUS RSH, Thermo Fisher Scientific). The specification of column used in GC-MS was TR5MS, 0.25mm ID, 0.25µm, 30m dimension. The temperature of ion source in DSQ II was maintained at 2000°C. Initial temperature in Trace GC Ultra was set to 400°C with hold time of one minute. Temperature in the inlet and MS transfer line was maintained at 207°C and 265°C, respectively. Full scanning mode was used at a scan rate of 500 amu/s and mass range between 15 to 120. Manual injection of sample was done in the sampling port in an air tight syringe and 50 µl volume of sample was injected. Pre-injection and post injection flush was given using nitrogen gas to avoid contamination. The time for pre-

injection and post injection flushing was 5 s and 30 s, respectively.

Preparation of edible film containing *C. quadrangularis* extract:

Edible coating solution was prepared by following the procedure mentioned by Hanumantharaju et al (2018) using starch (3, 4 and 5%), glycerol (20, 30 and 40% for each gram of starch) and extract (5, 10 and *C. quadrangularis* 15%). The 40±1.5 ml of film forming solution was poured into 14 cm diameter borosil petri plates and plates were placed in a ventilated hot air oven at 65 degree C for 3-4.5 h. After complete drying of these films, they were removed from the petri plates using scalpel and forceps. Prepared film samples were packed in polythene sheets and stored in an air tight container to prevent moisture absorption.

Edible Film Properties

Film thickness:

Hand-held micrometer (Mitutoyo micrometer) was used to measure the Film thickness at different parts of the film.

Opacity:

A Hunter lab flex colorimeter was used to measure the opacity of the prepared film. The colorimeter was calibrated using standard white and black background. Opacity of the prepared films was calculated according to the following equation (Fadini et al 2013)

Tensile strength:

Tensile strength of the film was determined using Universal testing machine (Lloyd instruments LRX plus, UK) and standard procedure was followed as prescribed by Matta et al (2015).

Water vapor permeability (WVP):

Permeation cells which is filled with a great amount of anhydrous silica gel (RH = 10%) provided with air gap of less than 6mm, was sealed by the prepared film using parafilm. Then the prepared permeation cells were placed in desiccators with a constant RH (52%) and temperature (30 C). Initial weight and weight of the film ° for every 2 h for 24 h were measured. The changes in their weight recorded was plotted against time. WVP was determined as mentioned by Nouri and Nafchi (2014).

Antimicrobial property:

The inoculum for the *E. coli* experiment was prepared in fresh Nutrient broths. The standardized inoculum was inoculated in the Petri plates prepared earlier and sterile

swabs were used to spread the culture on top of petri plates to ensure uniform spreading of culture after which the inoculums were left to dry at room temperature with the lid closed. The developed different composite film discs (3 cm diameter) of each treatment were placed on the surface of the previously inoculated agar culture media. Petri dishes with microorganism and discs of different composite films were incubated at $35\pm 1^{\circ}\text{C}$ for 24 h to allow the diffusion of antimicrobial compounds from the film. The antimicrobial activity of developed composite film was determined by measuring the inhibition zone around (Horita et al 2016).

Statistical analysis:

A box-behnken statistical tool under response surface methodology was selected, because of its best fit for three factorial full design and estimation under quadratic model.

RESULTS AND DISCUSSION:

GCMS analysis of the stem *Cissus quadrangularis* extract:

The main components present in the *C. quadrangularis* stem extract were phenolic compounds octadrine, piperidinol, phytol, phenylephrine benzenediol, 3-Ethoxyamphetamine and 1-Guanidinosuccinimide are the precursors for Vitamins E and K1, and common antioxidants. Phenolic compounds are also found to be an effective vaccine adjuvant with no adverse auto-immune effect. Similarly, Shah (2011) also confirmed the presence of phytochemicals in the extract.

Film thickness and opacity:

The thickness of the films was in the range of 79 to 136 μm . Opacity of the films was in range of 20.31 to 30.45 %. The starch has significant effect on increasing the opacity (Fig 1). . High concentration of starch opens the glucosides rings, this branched glucoside rings restricts the passage of light through the film (Fadini et al 2013). The increase in glycerol content increases the film opacity due to, polymer matrix formed by the addition of glycerol which hinders the light to pass through. The addition of plant extract significantly increased the film opacity due to the phenolic compounds in the extract which absorbed most of the light. Intermolecular interaction of the chemical compounds in the extract also hindered the light to pass through.

Tensile strength:

Tensile strength is the maximum tensile stress that the film can sustain when tensile forces are being exerted on the film continuously (Fig 2). . Film with 5,20 and 10

percent of starch, glycerol and plant extract showed the highest tensile strength (5.49 MPa). The film with 3, 40 and 10 percent starch, glycerol and plant extract showed the lowest tensile strength (0.98 MPa). Films 498 K.N. Hanumantharaju, K. Thangavel, D. Amirtham and C. Indu Rani .Opacity of the films at varying concentration of starch, glycerol and stem extract *C. quadrangularis*. Tensile strength of the films at varying concentration of starch, glycerol and stem extract *C. quadrangularis* with high starch concentrations showed high tensile strength as compared to the lower starch concentration films. This may be due to the availability of starch molecules for intermolecular bonding within the film. The tensile strength of the film decreased significantly with addition of plasticizer and plant extract. This may be due to the intermolecular forces which led to the phase separation between the molecules, decreasing the tensile strength of the film.

Antimicrobial property:

Disk diffusion assay revealed that specimens with 15 percent plant extract concentration showed anti-bacterial effect against Film with 3, 30 E-coli . and 15 percent starch, glycerol and plant extract showed higher inhibition zone of 1.5mm. Similarly, minimum inhibition zone of 0.5 mm was observed in 5, 30 and 15 percent starch, glycerol and plant extract. The studies confirmed the presence of antimicrobial compounds in the extract, and also the active compounds are effective against microbial growth at higher concentration. Kaya (2018) observed that, *Pistacia terebinthus* seed extract incorporated edible chitosan-based films showed inhibition zone against gram-negative bacteria such as *P. microbilis*, *P. vulgaris*, *P. aeruginosa* *E. coli* and Antimicrobial activity of the film might be due to presence of flavonoids and poly-phenolic compounds in the plant extract. The best fit quadratic equation (0.821) is as follows $R = s. 2$ Inhibition zone (mm) = $-0.12 * A^2 - 0.013 * B^2 + 0.54 * C - 0.038 * A + 0.038 * B + 0.54 * C - 0.25 * A * C - 0.025 * B * C$..(5) The numerical optimization by the Box Behnken method gave higher desirability for the edible coating formulation containing 3.33% starch, 20.00% glycerol and 15.00 % *Cissus quadrangularis* stem extract

CONCLUSION:

This study confirmed the presence of phytochemicals in the stem extract. The film prepared at *Cissus quadrangularis* different concentration of starch, glycerol and *cissus quadrangularis* stem extract have remarkable effect on EF properties. In this film formulation increase in starch concentration significantly increased the opacity,

TS and WVP. Increase in glycerol content increased opacity, WVP and WS, and decreased the TS of the film. Increased plant extract concentration decreased the TS of the EF. Opacity and WVP of the prepared EF samples were increased with increased plant extract concentration. Films with 15% plant Fig. 3. Water vapor permeability of the films at varying concentration of starch, glycerol and stem *Cissus quadrangularis* extract extract showed antimicrobial property on bacterial. *E. coli* In future these films can be used for fruits and vegetable as an antimicrobial packaging material.

CHAPTER-3

AIM AND SCOPE:

AIM OF THE PROJECT:

- Generally, edible films and coatings function as a barrier against gases or vapor and as carriers of active ingredients, such as antioxidant, flavours, fortified nutrients, colorants, antimicrobial agents or species.
- The purpose of edible films is to provide mechanical integrity or handling characteristics of the food, and a selective barrier to various gases, moisture, aroma, and lipids.
- Besides their barrier properties, edible films and coatings may control adhesion, cohesion, durability, and improve the appearance of coated foods.

There are several advantages associated with an edible coating like

1. They may be eaten by the consumer along with food.
2. Their use could reduce the waste and solve the solid disposal problem.
3. They could enhance the organoleptic, mechanical or nutritional properties of food.
4. They can reduce the cost by utilizing by products e.g., whey.

SCOPE OF THE PROJECT:

The scopes of the project are

- Water soluble within 24 hours.
- This process doesn't require any recycling or landfills.
- Edible food packaging eliminates the typical waste and polymers.

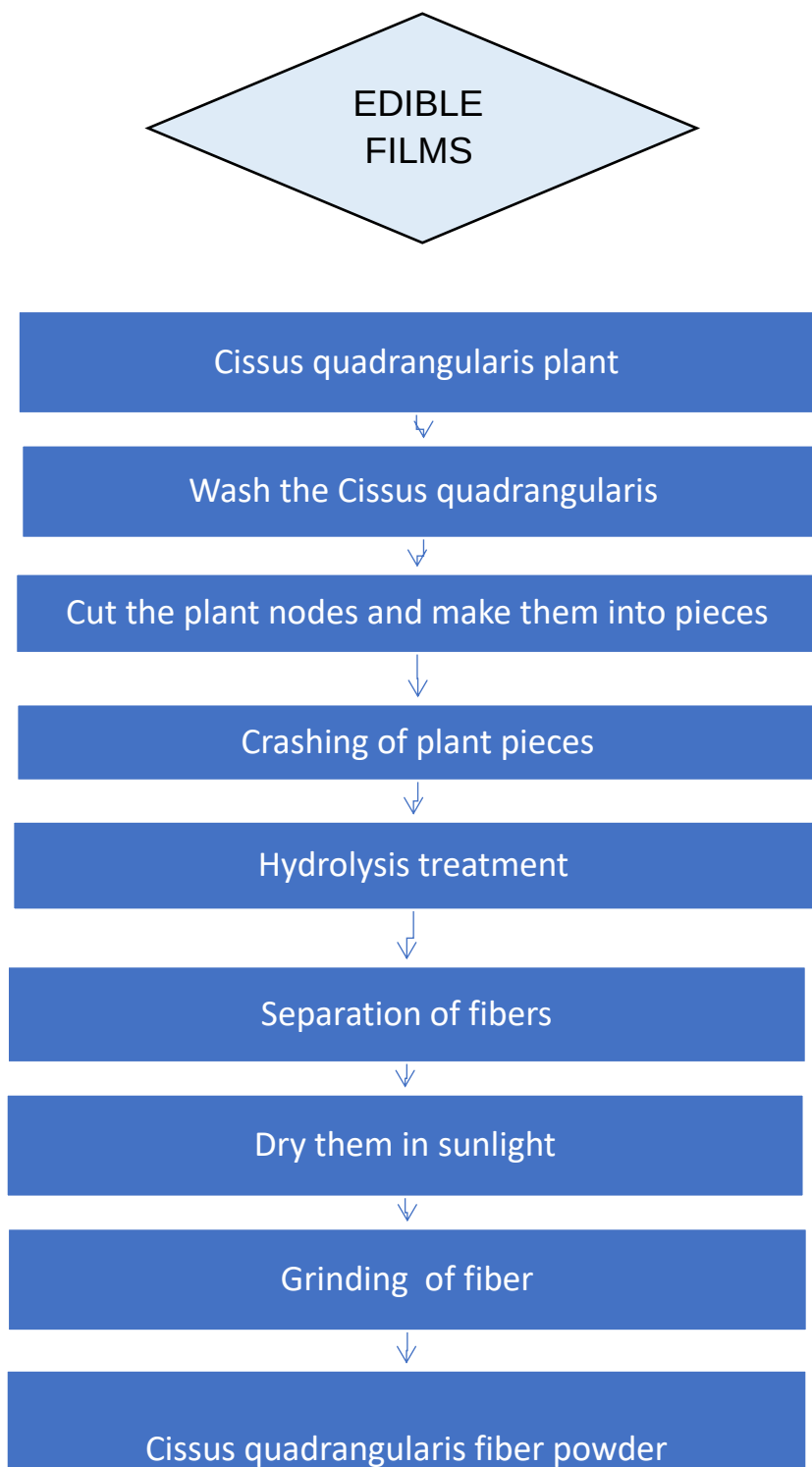
- Most of the edible packaging can be eaten or composted, biodegradable.
- Thus it enhancing safety and nutritional property.
- Film can work as carrier antimicrobial or antioxidant agents.

CHAPTER-4

MATERIALS AND EXPERIMENTAL METHOD:

METHODOLOGY:

Block diagram:



- We bought 5kg of *Cissus quadrangularis* from local market and nearby garden.



- As those *Cissus quadrangularis* contains mud on it, they should be washed thoroughly before processing.



- After washing them, the nodes of the plant were cutted into pieces. Thus to enhance the degradation process those pieces are slightly crashed with wooden hammer.



- Those pieces were soaked in the water (Hydrolysis Treatment).



- The set up was left undisturbed for a week so that the upper layer of *Cissus quadrangularis* were decayed and the fibers are started to separate.



- After a week of Hydrolysis treatment the fibers are separated. Those fibers are separated manually with proper protection by wearing mask and hand gloves.



- Those separated fibers are transferred to a dry cloth and set them under sunlight to absorb the moisture and further drying was done by hot air oven.



- After drying, those fibers are further cut into pieces to exhibit in the powder form.



- Then we transfer all those fibers into a mixer or a ball miller to grind them well into fine powder.



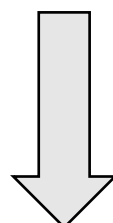
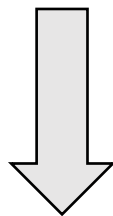
- After grinding them , finally we receive a fine fiber powder (Cissus quadrangularis) which is the major ingredient used in preparation of film.



PREPARATION OF FILM:

- + In a glass container, measure of 200ml of water and 2g of potato starch was mixed well.
- + Meantime the pan was pre heated with water at low flame and a steel bowl was kept over the pan. Which is nothing but double boiling method(DBM).
- + Those mixture of water and potato starch was transferred to the steel bowl.
- + It is stirred slowly at low flame for 5 minutes.
- + Then the fiber powder was added into the mixture of potato starch and water in the bowl.
- + To that of 2ml of glycerin is also mixed.
- + The mixture was stirred continuously for 20mins until they appear in viscous form.
- + Finally, transfer the viscous mixture on a stick file and let them dry.
- + After drying the film is appeared in sheet form.
- + Those dried film are tested for mechanical properties like shelf life, elongation and tensile strength.

HERE COMES THE PICTURE REPRESENTATION OF THE PROCESS:





MATERIALS REQUIRED:

The various materials used are

- *Cissus quadrangularis*
- Gloves,mask
- Potato starch
- Glycerin
- Scissors
- Dry cloth
- Hot air oven
- Sealed covers
- Mixer or ball milling
- Steel bowl
- Tightly sealed utensil

CHAPTER-5

RESULT AND DISCUSSION

RESULT:

- ❖ Thus, we successfully prepared the film with the help of extracted *Cissus quadrangularis* fiber powder.
- ❖ These film used to wrap the product which has many health benefits.
- ❖ They usually helps in increasing the shelf life of a particular product





DISCUSSION:

Since people have a hesitation to eat a package, these edible films can be supplied for low cost at initial time. Food packaging is one of the important things that are done by different materials. Plastics over the last two decades, with annual growth of 5%, are the second most widely used materials for packaging food, paper, and paperboard. Oil-based plastic materials such as polyethylene, polypropylene, polyamide, despite the environmental problems, are used extensively for packaging due to its flexibility, transparency, and stability. However, despite good properties, their use and accumulation cause serious problems. Starch is a good alternative to petroleum-derived polymers due to its easy availability, food contact, low cost, and digestibility. However, the use of starch in packaging also has problems. For

example, highly water-starch and its film properties are heavily dependent on moisture content and also have relatively poor mechanical properties. Its films are very fragile and resistant to oxygen permeation. In a study, the combination of PLA and starch with appropriate properties with suitable packaging properties was obtained and the results showed that different proportions of PLA-starch, (20-80 or 30-70) with epoxy soybean oil or malic a hydric, mechanical resistance, provides a very good prevention of water vapor and gases as well as good flexibility.²⁹ Khan et al., used thermoplastic material due to their intermolecular forces and hydrogen bonding starches, called thermoplastic starches (TPS), which increases the flexibility of starch, causing the stability of starch is below its degradation temperature. TPS is a very promising product for biodegradable plastics than biodegradable plastics.³⁰ Corn starch was applied as a polymer matrix for the increment of antimicrobial packaging applying the way of casting and applies of Pittides Nisin or Pediocin to preserve food. Halloysite nanoparticles were selected to enhance the film. The results showed that the adding of Nisin and Piodosine peptides in starch films to the creation of active packaging substances with antimicrobial activity against L. Monocytogenes and C. Perforation.³¹ Biodegradable starch/clay nanocomposite films were also used to pack foods. Montmorillonite nanomaterials (homogeneously distributed in various starch-based substances) were obtained by polymer melt processing methods. The structural and mechanical features of nanocomposite films were examined and the findings of the clay particle increase impact on the modulus and the starch strength revealed starch.³² Starch and starch derivatives, Polyhydroxylbutyrate (polylactic glycolide), have high properties that are compatible with antimicrobial agents for packaging. PHB, starch, and PLGA have unique properties in the fields of food, cosmetics, pharmaceuticals as well as various composites. Starchy films were mixed with chitosan and potassium sorbate compounds and active films used to inhibit E. coli growth and S Aureus, as well as the deterrence properties of the films.³³ An environmentally friendly biodegradable nanocomposite was made using potato starch and pineapple leaves. Due to the fiber structure and the dispersion of nanofibers on the starch field, the properties of nanocomposites increase after reinforcement to 3% by weight of cellulosic nanofibers, but in loading more filler, these properties are likely to decrease due to the density of nanofiber. The permeability of water vapor, water absorption and absorption of moisture nanocomposites were investigated and it was found that the

inhibitory properties increased significantly. The good dispersion and good adhesion of nanofibers to starch is the same structure of polysaccharides in both phases.³⁴ Jang et al., (2017) prepared silver and starch nanoparticles for one-stage coating using ultrasound and a mixture of starch, silver nitrate and distilled water, which used starch as an environmentally friendly and inexpensive agent. Ultraviolet-neomorphic and transient electron microscopy showed that the single-phase process was effective for the synthesis of starch-coated coatings with silver nanoparticles.³⁵ Jang et al., (2018) used synthetic zirconium and silver nanoparticles and starch (chitosan: starch-silver nanoparticle) for antimicrobial coating applications. Besides, the effects of different ratios of chitosan and silver nanoparticles and starches were studied on different study features such as mechanical features, water and oil opposition, and antimicrobial function. The findings revealed which the study covering features rely severely on the compound of these three substances, and then covering with a ratio of 1 to 9 shown good mechanical features and excellent resistance features to water and oil.

CHAPTER-6

CONCLUSION:

Edible films and coatings are promising systems for the improvement of food quality, shelf life, safety, and functionality. They can be used as individual packaging materials, food coating materials, and active ingredient carriers and to separate the compartments of heterogeneous ingredients within foods. Most biopolymers are relatively hydrophilic compared to commercial plastic materials. For industrial use, it is necessary to conduct scientific research to identify the film-forming mechanisms of biopolymers in order to optimize their properties. Various practical studies have been conducted to investigate the commercial feasibility of edible films and coatings, including new process evaluations, determining safety and toxicity, regulatory assessment, and consumer studies. However, it is expected that more studies will address the practical applications of edible films and coatings within the food industry. Contaminants from petroleum-based polymer materials have focused on the production of biodegradable polymers. The global trend of research and industry

development is towards the use of biomass and renewable and environmentally friendly materials. Starch is one of the most abundant and cheapest substitutes for petroleum products and is now considered by researchers. Extending the use of starch structure techniques and the use of this material in combination with other materials to reduce the inherent weaknesses of this natural polymer has led to its further development in various industries, specially packaged industries. It is expected that in Iran, taking advantage of global experience, the production and use of starch will be developed as an important raw material in various industrial sectors. Due to novel research on the application of bio-degradable biofilms in the packaging industry of food, starch is one of the most promising and promising sources. Different needs in the food packaging industry for bio-films have led to the diverse sources of starch being studied because each source has its specific characteristics. While various starch components themselves are effective on the characteristics of the film, they produce different behavioral characteristics. To adapt the starch to create an interesting film, it can be revised by applying different substances. It creates films with interesting features, is free from improper chemicals during the film. Another new approach to improving the mechanical features and starch permeability is the application of different polymers with starch in film process that has the main impact on the improvement of the features of starch-based films; it may also have other features, such as antimicrobial features. The many novel studies on starch composite films are the use of bio-nano-composites that can have a good impact on the features of these films.

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EDIBLE FILMS USING CISSUS QUADRANGULARIS



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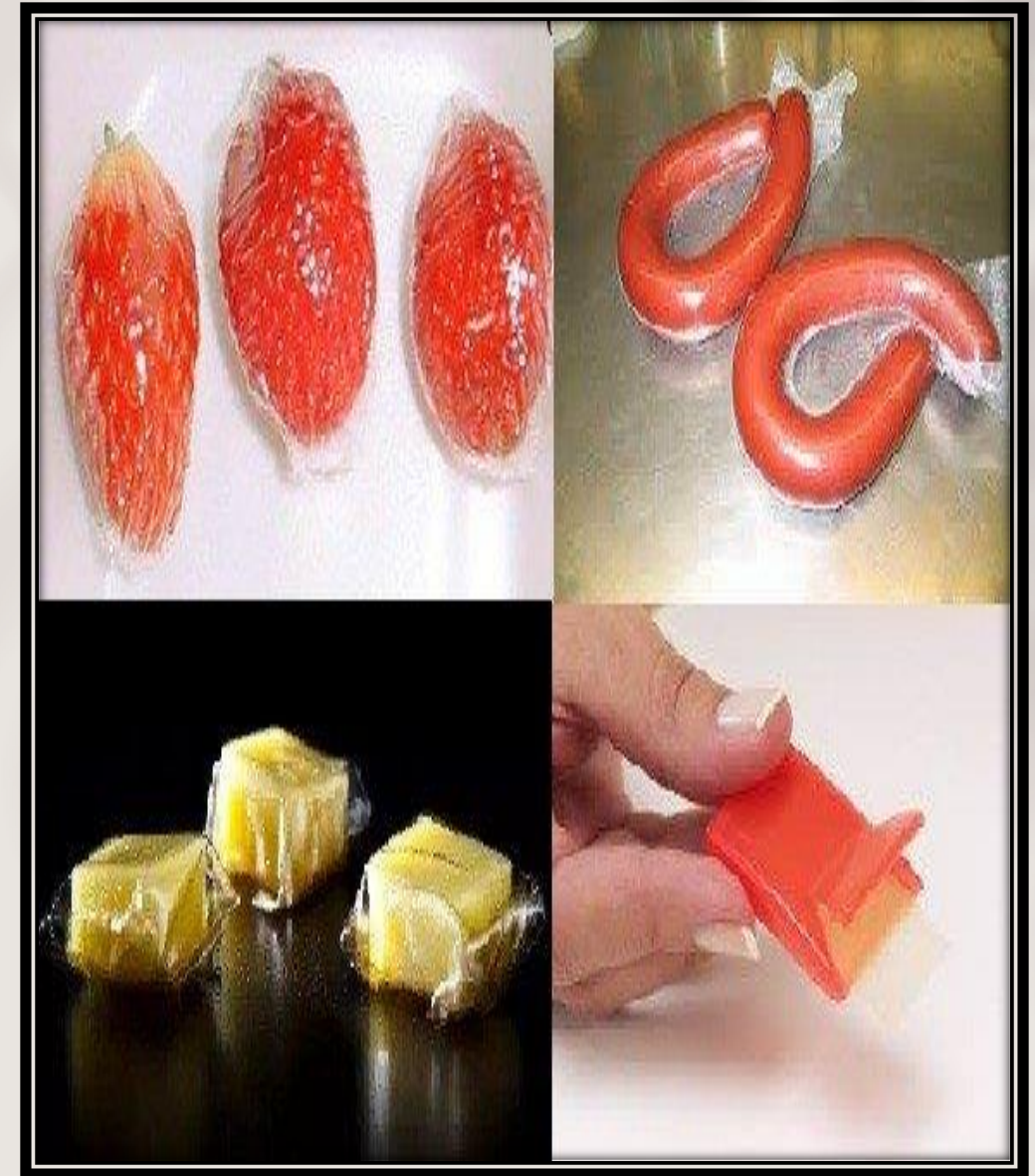
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PREPARATION OF STARCH BASED EDIBLE FILMS INCORPORATED WITH CISSUS QUADRANGULARIS FIBER

INTRODUCTION

Edible films and coatings are thin layers that are coated on the surface of food products. Beyond those coming from the conventional ones, the main functions of this type of packaging are edibility and biodegradability. Some of the edible food coating applied are starch, whey, beeswax and gluten. In addition, films are water soluble and dissolve by almost 90% in 24 hours. Although many bio-films including whey gluten, were found suitable for packaging, none of them ever tested so far add better outcomes than the existing bio-films and therefore bio-films are commercially not viable to date. Hence in this project work, a novel bio-film additive derived from naturally available herbal species known as *Cissus quadrangularis* has been proposed. One major advantage of using edible films and coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes. These films are environment friendly.



PROCEDURE

STEP 1: We bought 5kg of *Cissus quadrangularis*.



STEP 2: Wash the *Cissus quadrangularis* before processing.



STEP 3: Cut the nodes of the *cissus quadrangularis* and crash them into pieces, to enhance the degradation.



STEP 4: Those fibers underwent hydrolysis process(soaking).



STEP 5: The set up was left undisturbed for a week so that the *cissus quadrangularis* were decayed and the fibers are separated.



STEP 6: After a week of treatment fibers were washed and taken separately



STEP 7: Those separated fibers are dried under sunlight and further drying was done by hot air oven



STEP 8: After drying, those fibers are further cut into pieces to exhibit in the powder form



STEP 9: Then we transfer all those fibers into mixer to grind them well

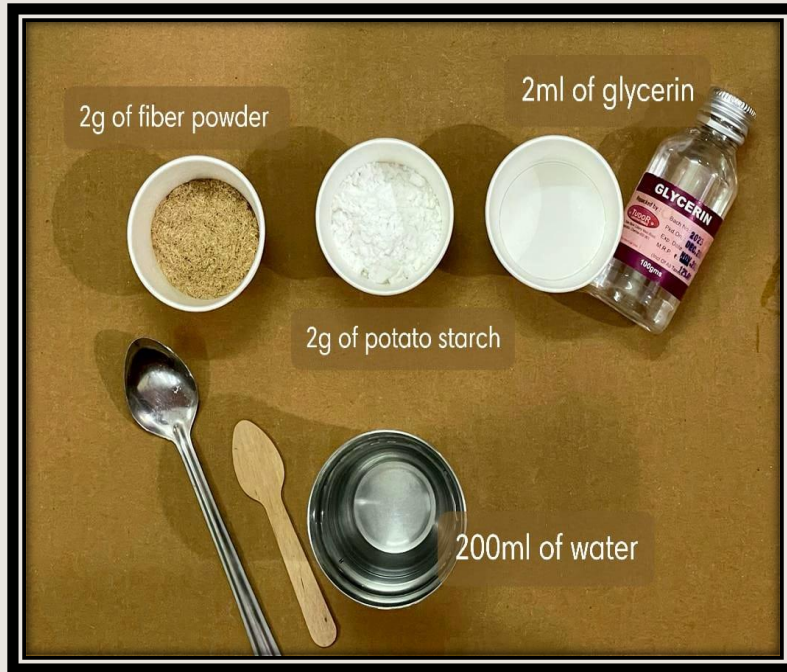


STEP 10: After grinding them , finally we receive a fiber powder (Cissus quadrangularis).



STEP 11: Preparation of film

- ❖ For 200ml of water, 2g of potato starch and fiber powder was added in a container. Stir them for 5min at low flame.



- ❖ To that 2ml of glycerin is mixed and stirred continuously for 20min until they appear in viscous form



- ❖ Transfer them on stick file and let them dry



FINAL PRODUCT

Film was prepared successfully ,



THANK YOU

