SHELF LIFE ENHANCEMENT OF FRESH FRUITS AND VEGETABLES USING PAPAYA LEAF EXTRACT INCORPORATED EDIBLE COATINGS

B. Tech Research Project Proposal Submitted by

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ABSTRACT

The effect of protein and polysaccharide based composite coating incorporated with the antimicrobial extract of papaya leaves on the shelf life of grapes and cucumbers were studied. Fresh grapes and cucumbers were coated with a three different composite coatings i.e. pectin with soya protein (PS), pectin with whey protein (PW) and pectin with casein(PC) with incorporation of papaya leaf extract and compared to samples coated with PS, PW and PC without papaya leaf extract. Another bilayer coating was applied on the sample which consisted of soya oil emulsion in pectin but the oil and pectin layer separated and a continous coating was not formed. All these samples were kept at room temperature along with uncoated sample to study the enhancement of shelf life.The result showed that the edible coatings proved to extend the shelf-life of fresh cut apple slices by decreasing delaying changes in color and water loss during storage. Among the coatings tested PS coatings were more effective than PC coatings, with antimicrobial agent incorporated coatings being more effective than their non-antimicrobial extract incorporated coatings. Papaya leaf extract incorporated PC coatings were more effective in preventing moisture loss while papaya leaf extract incorporated PS coatings improved the overall shelf life by preventing spoilage to a greater extent.

KEY WORDS: *edible coatings, papaya leaf extract, pectin, soya protein, casein*

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INTRODUCTION

- Fruits and vegetable offer shoppers profoundly nutritious and convenient items while keeping up the freshness of the non-prepared items. Consumers ordinarily judge the nature of fresh fruits and vegetables based on appearance and freshness at the season of procurement. One strategy for expanding post-harvest time span of usability is the utilization of the edible coatings. Consumable films and coatings have gotten extensive consideration as of late in light of their favorable advantages including use as eatable packaging materials over engineered synthetic films. [1] The principle advantage of edible coatings over conventional synthetics is that:
- They can be consumed with the packaged products
- There is no package to dispose even if the films are not consumed
- They contribute to the reduction of environmental pollution. [2]

NEED FOR EDIBLE COATINGS

Post-harvest losses of fruits are serious problem because of rapid deterioration during handling, transport and storage in tropical regions. As a result, large volumes of fruits and vegetables are wasted every year. The post-harvest losses affect the livelihoods of nearly one-third of the population, the majority of who are small holder farmers. Thus, need for new solutions for reducing post-harvest losses by increasing the shelf life of fruits and vegetables during transportation and storage has become very important and edible coatings can play a major role in meeting this feat. [3]

EDIBLE COATINGS

Edible films are thin layers of edible material applied to the item surface along with or in place of natural defensive waxy coatings and can possibly diminish exchange of moisture, limit oxygen entrance, bring down the rate of respiration, reduce ethylene generation, seal in flavor volatiles, and carry additives that impede microbial growth and discoloration.[4]

Fresh fruits and vegetables keep on respiring even after harvesting and use all the oxygen inside the product, which isn't as easily replaced by edible films. This leads to the accumulation of the carbon dioxide produced because it cannot escape through the edible coating. As a result the fruits and vegetables will move to partial anaerobic respiration that doesn't require as much oxygen as aerobic respiration(1– 3%). [Park et al; 2000] With less oxygen, the production of ethylene (which quickens the maturing process) is disturbed and physiological loss of water is minimal and their shelflife nearly doubles. [2]

The properties of edible coatings include:

- The covering ought to be water-safe so it stays flawless and covers an item satisfactorily, when connected.
- It ought not to exhaust oxygen or accumulate unnecessary carbon dioxide.
- It ought to diminish moisture permeability.
- It ought to enhance appearance, keep up the structural integrity, enhance mechanical handling properties, carry active ingredients (vitamins, antimicrobial compounds etc.) and hold volatile flavor components.
- It ought to never meddle with the
- organoleptic and chemical properties of fruits and vegetables vegetable and not confer undesirable odor.
- It ought to have low viscosity and be reasonably priced as well as easily available. [5]

Now a day's various types of edible coatings are available. They can be classified on the basis of basic structural component. The edible coatings are mainly divided into three classes on the basis of function:

- Hydrocolloids: e.g., polysaccharides, proteins and alginate.
- Lipids: e.g., fatty acids, acryl glycerides and waxes.
- Composites: e.g., protein/protein, polysaccharides/protein, lipid/polysaccharides [8]

Hydrocolloids: Hydrocolloids have hydroxyl groups and might be polyelectrolyte's, for example, Alginate, Carrageenan, Pectin, Carboxy Methyl Cellulose, Xanthan gum and Gum Arabic. By and large, all hydrocolloids have the properties to completely or partially dissolve in water and standard utilization of this is to increase the consistency and viscosity of the aqueous phase i.e., gelling and thickness agent.

The hydrocolloids are categorised into two classes-Polysaccharide-based, Protein-based. Polysaccharides based edible films having poor moisture hindrance properties, can be dissolved in water. However, it contains comparatively less oxygen permeability. Polysaccharides give crispiness, hardness, compactness, to the edible coatings. Polysaccharides comprise of polymer chain, having phenomenal gas obstruction properties, bringing in desirable modified atmosphere.

Soya protein

The protein percentage of soybeans (38-44%) is comparativelymore than the protein percent of cereal grain (8-15%). Most soy proteins (90%) are globulins, which can be fractionated into 2S, 7S, 11S and 15S according to their sedimentation coefficients. The strong charge and polar interactions between side chains of soy protein molecules limits segment rotation and molecular movement, which increase the stiffness, yield point, and tensile strength of soy protein films.

- The formation of films from soy proteins has been described as a two-step process involving:
  a. The application of high temperature to denature the protein structure, break the native disulfide bonds and expose sulfhydryl groups and hydrophobic groups.
  b. The formation of new disulfide, hydrophobic and hydrogen bonds.

Casein

Milk proteins can be ordered into two categories: casein and whey protein. Consumable protein films can be sourced by solubilization of various caseinates in water followed by application and drying. Caseinates films are produced using aqueous solutions because of their arbitrary coil nature. Molecular Interactions in the film lattice are likely to incorporate hydrophobic, ionic, and hydrogen holding. Caseinate films are transluscent and flexible, but have poor moisture barrier properties. Casein has been studied for the development of free-standing films and coatings on food items. [2]

Whey protein

Whey protein is a by-product of cheese processing and is especially rich in β-lactoglobulin. Whey proteins (20% milk proteins) when properly processed produce flexible yet fragile coatings. They have moderately high nutritional benefits and have been broadly studies as edible coatings. Whey protein is easily process able has some potential as exterior coatings, when mixed with gelatin, however suitable modification methodologies can be created to decrease moisture sensitivity. [4]

Composites Based Edible Coating

Composites or Multi component films and coatings contain combination of protein, polysaccharides and lipid-based material. According to Han et al. composites are divided into two categories given below

- Bilayer composites: The bilayer composites consist two layers combined with same or different coating materials [2]
- Conglomerates: The film comprising a conglomerate of lipid and hydrocolloid components where in the lipid component is derived from a dried fat composition.

As a general rule, **fats are used to reduce water transmission; polysaccharides are used to control oxygen and other gas transmission, while protein films provide mechanical stability**.

SOME PREVIOUS WORK

Anti-microbial activity of papaya leaf extract

It was reported that the extracts of papaya leaves could inhibit the growth of some bacterial pathogens. Papaya leaves were extracted by using maceration method and three kinds of solvents: ethanol and ethyl acetate. Papaya leaf and stem extracts were tested against both Gram positive and Gram-Negative bacteria such as Staphylococcus aureus, Streptococcus pneumonia, Bacillus cereus, Salmonella typhi, Escherichia coli and Pseudomonas aeroginosa by diffusion method. The extract demonstrated higher activities against all the Gram-negative bacteria than Gram positive bacteria tested, with the highest activity (16 mm zone of inhibition) demonstrated against Salmonella typhi. [18]

OBJECTIVE:

Nature is a very good chemist and we are learning from that and sometimes improving on it with new edible coatings that protect the quality and nutritional value of food.

So with this point in mind and also with the increasing awareness of the consumers towards "eating healthy" we have undertaken this project. The **main objective** of this project is to:

- BROAD OBJECTIVE

1. To enhance the shelf life of the product.
2. To provide an edible coating which self-perseveres and also add to the antimicrobial properties of the food.
3. To maintain freshness, flavor, aroma, texture and nutritional value of food products using biodegradable coating.

- SPECIFIC OBJECTIVE

1. To use different combinations of hydrocolloids and determine the best combination of composite layer for shelf life enhancement
2. To determine the efficiency of papaya leaf extract as an anti-microbial agent.
3. To improve the nutritional value of the product using nutritionally rich coatings.

4. To provide alternative materials for existing edible coating that is both, if not more, efficient and pocket friendly.

METHODOLOGY

A bilayer was prepared as a composite layer incorporated with antimicrobial agents as the first layer and a lipid based second layer.

First layer: A conglomerate of a polysaccharide and a protein component to form a film with improved elastic, and barrier properties.

Second layer: A lipid based bilayer to provide moisture barrier.

Samples choosen: grapes, cucumber

COMPOSITE LAYER COMBINATIONS (first layer)

- pectin-Soy protein
- pectin-whey protein
- pectin-casein

I. EXTRACTION OF ANTIMICROBIAL AGENT

1. Papaya leaf extract

The powdered leaves were soaked in distilled water and heated to extract the antimicrobial components into water. The mixture was the macerated and sieved using a muslin cloth. The extract obtained is then used as antimicrobial agent in the coatings.

II. FILM PREPARATION (first layer)

1. Whey protein films

Whey protein 5 % (w/w)* is slowly added to distilled water until the whey protein isolatehas completely dissolved. Solutions are placed in a water bath at 90 °C for 30 min to denature the protein completely. Solutions are then left undisturbed for coolingatambient temperature, and Glycerol (50%, w/w) was added to plasticize the films. [0zdemir; 2008]

2. Soy protein films

Soy protein isolates(minimum 90% protein content on dry basis) is added to distilled water along with constant stirring at a concentration of 5% along with 2.5% of glycerol. The solutions is heated at a temperature of 70 °C for 20 min in constant temperature water bath. The solution pH is then adjusted to 10±0.1 by. [ReihanehAhmadzadehGhavidel et al; 2013]

3. Pectin

Pectin (3%) is dissolved in DW at the temperature of 90°C. To this glycerol (2.5%), citric acid (1%) is added and mixed using a magnetic stirrer. After mixing the solution is allowed to cool.[Menezes; Athmaselvi K.; 2016]

4. Casein:

Exactly 10 g of casein or WPC was weighed and dissolved in 200 mL of warm distilled water. The pH of the film-forming solution was adjusted to 5.6 by adding 2 N NaOH solution. The film-forming solution was heated and stirred at 85 °C for 15 min. Plasticizer was added, and heating was continued for another five min. Potassium sorbate at 0.2 % (w/w) was then added, and the film-forming solutions were cooled to 40–45 °C. [McHugh et al. (1994)]

*in composite layer, the concentration are adjusted according to the combination used.

III. FILM PREPARATION (Second layer)

A lipid layer is coated on top of first dried, composite layer using vegetable soya oil

IV. SHELF LIFE STUDIES

The film coated samples are kept at room temperature and refrigeration temperature and the shelf life is studied.

FLOW CHART

Selection of samples

↓

Extraction of antimicrobial agent from papaya leaf

↓

Preparation of first composite layer with incorporation of suitable microbial extract

↓

Coating of layer on the sample surface by dipping/ brushing followed by immediate drying

↓

Application of second lipid based layer by dipping/brushing followed by immediate drying

↓

Storage of samples at refrigeration temperature and room temperature

↓

Shelf life analysis (analysisi of texture, colour changes, and growth or spoilage by any micro-organism)

PRETESTING

The pre-testing was carried out in Food technology laboratory at Bhaskaracharya College of Applied Sciences on 5 December, 2016 in the morning at 11:00am. The temperature of the lab was 21ºC. Fresh unbruised cucumber and cucumber was taken for edible coating.

TRIAL ONE

- **Pectin coating**

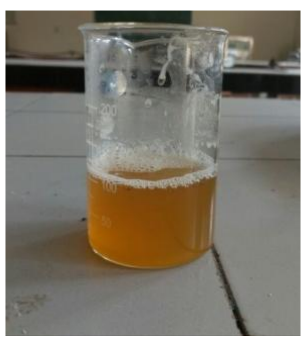
It was conducted using 5% pectin solution. Capsicum was dipped in the solution and immediately blow dried under the drier. The coating so formed was stable, continuous and elastic. It gave a glossier and thicker coating.

- **Soy protein coating**:

It was conducted using 7% soy protein solution. A good thin film was formed on the surface. There was no flaking and the film was continuous.

TRIAL TWO

A composite layer was formed using pectin and soya mixed in a ratio 4:3. It gave a good thin and continuous layer. Very little flaking was observed on the edges.



(a)

(b) (c)

Figure: (a) soy protein solution and (b) pectin solution (c) cucumber with composite coating of soya and pectin.

TRIAL THREE

In trial three the edible coatings were made as per the procedure mentioned and then mixed together in the ratio of 1:1.The mixture was then allowed to cool to room temperature to allow the formation of proper network. It was observed that when whey protein solution was mixed with pectin, there was immediate coagulation of the mixture. The edible coating was then applied by dipping in case of grapes and brushing in case of cucumber along with continuous drying under a hand drier.

TRIAL FOUR

Trial four was conducted in similar manner as trial three but there was an additional incorporation of the papaya leaf water extract after mixing of the pectin along with protein solution and before cooling.

The shelf life of the coated sample was studied at room and refrigeration temperature in terms of change of color, texture and overall appearance.

TRIAL FIVE

Procedure is same as above except that soyabean refined oil was added to the solution while being heated. The resulting solution after cooling had two layers not distinctly separated that of pectin-soy gel and oil layer at the top. On application of coating there was no continoius film formation. This might have happened because oil hinders gelatinization process. Cucumber coated with this solution was of bad quality, flaky and had grainy hand feel as the oil solidified on the surface of the sample due to drying and was sticking to hands.

OBSERVATION

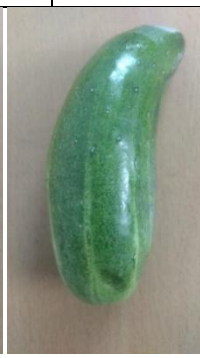
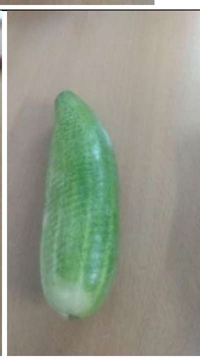
COATING CHARACTERISTICS

The pectin+ soya coating was slightly turbid. It formed an elastic, continous and shiny coating. The coating was slightly thick, but was stable and no flaking was observed.

The pectin+casein coating was completely transparent. It formed a more elastic, thin and uniform coating. It gave a better glossy finish to the sample surface. It was stable with no flaking.

On mixing whey with pectin the protein coagulated and no coating was formed.

SHELF LIFE ANALYSIS

	With papaya leaf extract	Without papaya leaf extract	With papaya leaf extract	Without papaya leaf extract	CONTROL
Day 1 Soya + Pectin					
Day 1 Casein+ Pectin					

DAY 1		Colour
CONTROL		Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm
SOYA +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance
	WITHOUT PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance
CASIEN +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance
	WITHOUT PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance

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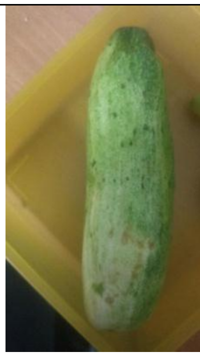
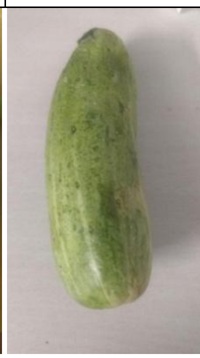
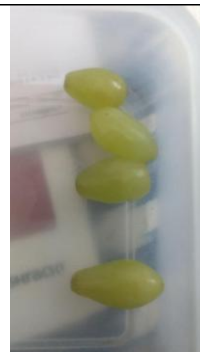
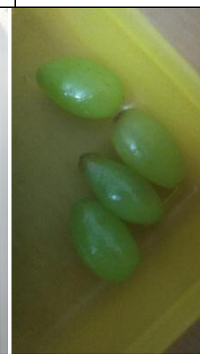
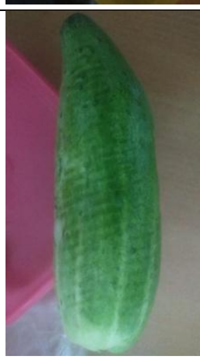
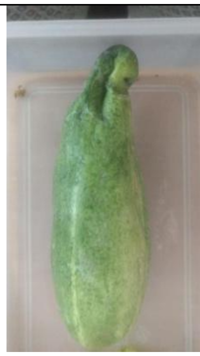
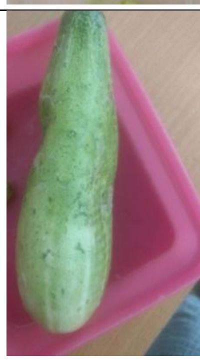
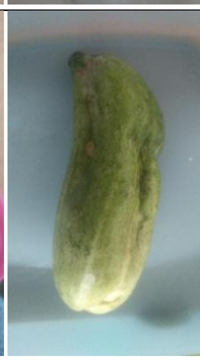
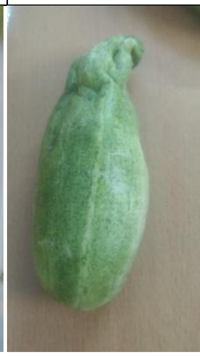
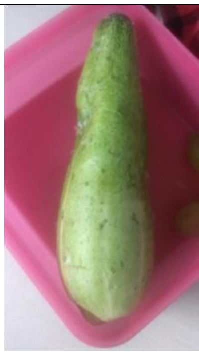
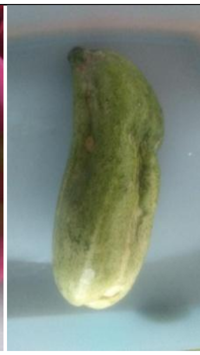
	With papaya leaf extract	Without papaya leaf extract	With papaya leaf extract	Without papaya leaf extract	CONTROL
Day 2 Soya + Pectin					
Day 2 Casein+ Pectin					

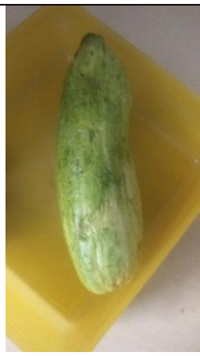
DAY 2		Colour
CONTROL		There was no visible changes in colour and it was still fresh Grapes were of bright green colour with no discolouration There was loss of firmness and shrinkage was visible on the samples
SOYA +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour with no change in colour Grapes were of bright green colour with no discoloration Smooth texture, firm, glossy appearance, no shrinkage
	WITHOUT PAPAYA LEAF EXTRACT	Cucumber was dark green colour with no change in colour Grapes were of bright green colour with no discoloration Smooth texture, firm, glossy appearance, no shrinkage
CASIEN +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour with no change in colour Grapes were of bright green colour with no discoloration Smooth texture, firm, glossy appearance, no shrinkage
	WITHOUT PAPAYA LEAF EXTRACT	Cucumber was dark green colour with no change in colour Grapes were of bright green colour with no discoloration Smooth texture, firm, glossy appearance, no shrinkage

	With papaya leaf extract	Without papaya leaf extract	With papaya leaf extract	Without papaya leaf extract	CONTROL
Day 5 Soya + Pectin					
Day 5 Casein+ Pectin					

DAY 5		Colour
CONTROL		Slight loss of color in cucumber Loss of colour with brown discoloration around the pit in grapes There was loss of firmness and shrinkage was visible on the samples
SOYA +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance
	WITHOUT PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour with slight discoloration around the pit Smooth texture, firm, glossy appearance, shrinkage observed
CASIEN +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance
	WITHOUT PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour with slight discolouration Smooth texture, firm, glossy appearance

	With papaya leaf extract	Without papaya leaf extract	With papaya leaf extract	Without papaya leaf extract	CONTROL
Day 7 Soya + Pectin					
Day 7 Casein+ Pectin					

DAY 7		Colour
CONTROL		Green color lost with appearance of pale yellow colour on cucumber Loss of colour with brown discoloration around the pit There was loss of firmness and sample was dried from one end
SOYA +PECTIN	WITH PAPAYA LEAF EXTRACT	Cucumber was dark green colour Grapes were of bright green colour Smooth texture, firm, glossy appearance, shrinkage observed
	WITHOUT PAPAYA LEAF EXTRACT	Slight loss of colour in cucmber Grapes were of bright green colour with slight discoloration around the pit glossy appearance, shrinkage visible
CASEIN +PECTIN	WITH PAPAYA LEAF EXTRACT	Slight loss of colour in cucmber Grapes were of bright green colour Smooth texture, firm, glossy appearance, shrinkage observed
	WITHOUT PAPAYA LEAF EXTRACT	Slight loss of colour in cucmber Grapes were of bright green colour with slight discoloration around the pit glossy appearance, shrinkage visible

	With papaya leaf extract	Without papaya leaf extract	With papaya leaf extract	Without papaya leaf extract	CONTROL
Day 9 Soya + Pectin					

DAY 9		Colour
CONTROL		Complete loss of colour, complete drying at one end Loss of colour with brown discoloration around the pit There was loss of firmness and sample was dried from one end Mould growth was seen
SOYA +PECTIN	WITH PAPAYA LEAF EXTRACT	loss of colour in cucmber Grapes were of yellowish greencolour with slight discoloration around the pit shrinkage observed
	WITHOUT PAPAYA LEAF EXTRACT	Yellowish color developed in cucumber Extreme discoloration, completely spoiled Complete loss of texture
CASIEN +PECTIN	WITH PAPAYA LEAF EXTRACT	loss of colour in cucmber Grapes were of yellowish greencolour with slight discoloration around the pit shrinkage observed
	WITHOUT PAPAYA LEAF EXTRACT	Yellowish color developed in cucumber Extreme discoloration, completely spoiled Complete loss of texture

RESULT AND DISCUSSION

From the above observation a number of things can be concluded.

In case of soya and pectin coating with and without antimicrobial agent both the cucumber and the grapes were in acceptable condition till day 3 with no visible change in the colour or texture of the product. On day fourth there was slight shrinkage visible on one end of both the cucumbers while only slight color change was observed in the grapes coated with the coating not containing the antimicrobial agent. Also the grapes were a little less firm which may be due to some moisture loss. Prominent shrinkage and color change was visible on the 6th day with the grapes becoming brown around the pits and cucumber losing their bright green color and taking a slightly yellowish appearance. Drastic color change was seen in grapes coated with plain composite layer without the antimicrobial extract. On day 8th the samples were completed unacceptable in terms of texture and overall appearance, but the grapes coated with antimicrobial incorporated coating still had there color intact while non antimicrobial coated grapes were completely spoiled in appearance.

On comparison with casein pectin coated sample a similar trend was observed but no visible signs of shrinkage were seen in the samples till the 5th day. It was also seen that the samples underwent discoloration at a faster rate than soya pectin coated sample and the non antimicrobial agent incorporated sample showing discoloration on the third day itself.

In case of the control (uncoated sample) loss of firmness and color could be seen from the second day itself. Also, the rate of shrinkage and color loss was accelerated in the uncoated sample, with the samples becoming completely unacceptable on the fourth day.

So, it can be clearly inferred that use of microbial extract in the coating prevents spoilage in terms of color, texture and microbial growth. The microbial extract incorporated coated samples had the highest shelf life, followed by samples coated with just the composite layer, while uncoated samples underwent degradation at a very fast rate having a shelf life of just one day.

Also, it can be seen that soya coating and casein coating were equally effective in preventing spaoilage and discoloration, while casein coating helped in perevention of shrinkage. This shows that casein forms a more continous layer that acts a batter barrier to water loss, while both soya and casein coatings act as oxygen/ gas barrier.

COST ANALYSIS

Cost per 100 gm

Pectin: Rs. 240

Soya protein isolate: Rs. 120

Casein protein isolate: Rs. 150

Glycerol: Rs. 50

Cost per 5 gm (i.e the amount required to make 100 ml of solution):

Pectin: Rs 12

Soya protein isolate: Rs. 6

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Casein protein isolate: Rs. 7.5

Glycerol: Rs. 1

Cost of 100 ml of each coating:

Pectin + soya + glycerol= 12+6+1= Rs. 19

Pectin + casein + glycerol= 12+7.5+1= Rs. 20.5

No. of cucumbers that can be coated with 100 ml of solution= approx. 10

Amount of grapes that can be coated with 100ml of solution= approx. 500gms

Therefore, cost of coating one cucumber is approx 1.9 rupees and half kg grapes is approx. 19 rupees for soya +prctin coating and Rs. 2/ cucumber and Rs. 20/ 500g of grapes for Pectin+ casein coating.

FACILITIES REQUIRED

Samples: Cucumber and grapes in good condition and without any infestation.

Chemicals: alginate, pectin, soya protein, whey protein, glycerol, citric acid, Akaline sodium salt, distilled water, ethanol, hexanol

Facilities required: Magnetic stirrer, water bath, gas stove, drier and weighing balance are the few facilities required. They are available in our labs and are present in good working conditions. The weighing balance is accurate and properly calibrated.

EXPECTED OUTCOME/ NATIONAL SIGNIFICANCE:

So from this project we are expecting positive results for:

1. Prevention of huge wastage of fruits and vegetables due to spoilage and improved economic benefits.
2. Easy availability of fruits and vegetables thus, reducing nutritional deficiency among population.
3. A new innovative edible coating with better flexibility and film forming ability
4. A better quality product.
5. A longer shelf life for fruits and vegetables.
6. Nutritional value addition because of nutritionally beneficial coating material.