PREPARATION, CHARACTERISATION AND EVALUATION OF ALGINATE FILMS MODIFIED BY ASCORBIC ACID AND CHARCOAL POWDER FOR FOOD PACKAGING APPLICATIONS

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ABSTRACT

SA is naturally occurring anionic polysaccharide widely known for its bio-degradability, low cost, bio-compatibility and low toxic nature which can be effectively used as packaging material for portioned products and charcoal powder is very known for its ethylene scavenging property. In this work, hydrogel films composed of ALG, honey, charcoal powder and ascorbic acid was prepared and characterised. The films were evaluated for contact angle measurement, FT-IR analysis, SEM analysis, swelling studies, DPPH radical scavenging assay and mechanical properties. The developed film is highly hydrophilic in nature and exhibited high antioxidant property. The antioxidant property of ALG, honey, ascorbic acid and charcoal based films are fruitful for the advancement in packaging and transportation of fresh cut fruits and vegetables in the present context.

INDEX

LIST OF SYMBOLS, ABBREVATIONS AND NOMENCLATURE

Symbols, abbrevations, nomenclature	Full name
ALG	Alginate
Ca	Calcium
$CaCl_2$	Calcium chloride
FT-IR	Fourier Transform Infrared Spectroscopy
Fig	Figure
SA	Sodium Alginate
SEM	Scanning Electron Microscopy
℃	Degree celsius

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1. INTRODUCTION

Fruits and vegetables are good source of vitamins and minerals including folate, Vitamin C, potassium etc. and so a diet rich in vegetables and fruits can lower blood pressure, reduce the risk of stroke and heart disease[1], prevent some type of cancer, lower risk of eye[2] and digestive problems. So intake of such food stuffs are inevitable in our daily life. Usually these products are transported by storing them in waxed card board boxes as well as plastic containers. Such packaging can't diminish the undesired physiochemical changes and prolong the shelf life of fresh cut produce during the storage or transportation period. Since the usage of plastics exceeds a million times annually it is very necessary to replace the conventional plastic materials due to their intense polluting character and non- renewable nature.

SA is a non toxic, bio degradable, bio compatible, cheap hydrocolloid having many applications in the food industry as a packaging material [3, 4]. Utilisation of appropriate ethylene scavenger like charcoal powder can reduce the rate of ripening of fresh fruits and vegetables [5, 6]. Honey can be used as a plasticizer [7, 8]. Antioxidants such as citric acid, ascorbic acid etc. [9, 10, 11] can delay rancidity and thereby increase the shelf life. Here we are developing an edible, natural food packaging film that can be used to pack and preserve fresh cut vegetables, fruits and also a film that can significantly reduce the ripening rate of fruits, which can create a welcome change in the food packaging field.

1.1 SA

SA is a naturally occurring anionic polysaccharide widely known for its bio-degradability, low cost, biocompatibility and low toxic nature with a wide range of applications such as packaging material for portioned products, limiting the dehydration of meat, as a thickening agent, in gel formation and as a colloidal stabilizing agent in the beverage industry, in the textile, pharmaceutical and paper industries and being used to obtain polymeric matrices [3, 4].

ALGs are isolated from the cell walls of brown algae [12, 13] like Laminaria digtata / Ascophyllum nodosum where they are found in the form of metal salts of organic acid. It can also be obtained by bacterial biosynthesis from Azotobacter and Pseudomonas exhibiting a more defined chemical structure and physical properties, when compared with the sea weed derived ALG [3]. The formation of strong gels or polymers with low solubility is due to its ability to react with polyvalent metal cations, especially with Ca ions [14]. This characteristic has led to the improvement of mechanical properties, barrier properties, cohesiveness and rigidity.

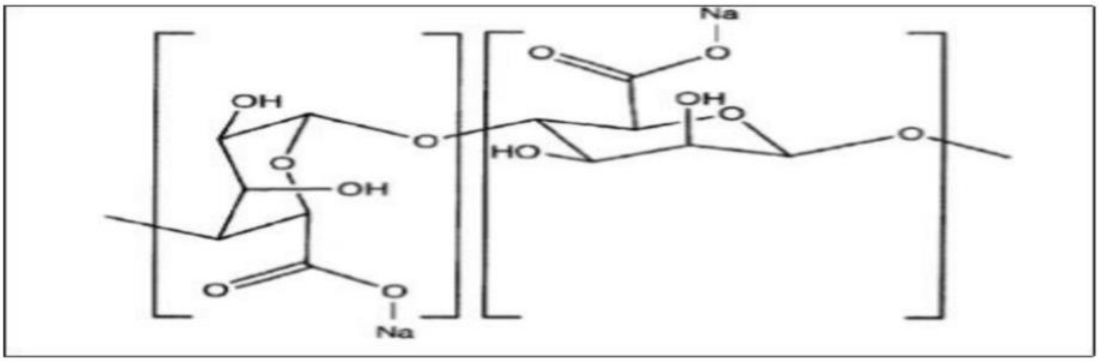


Fig 1. Structure of SA

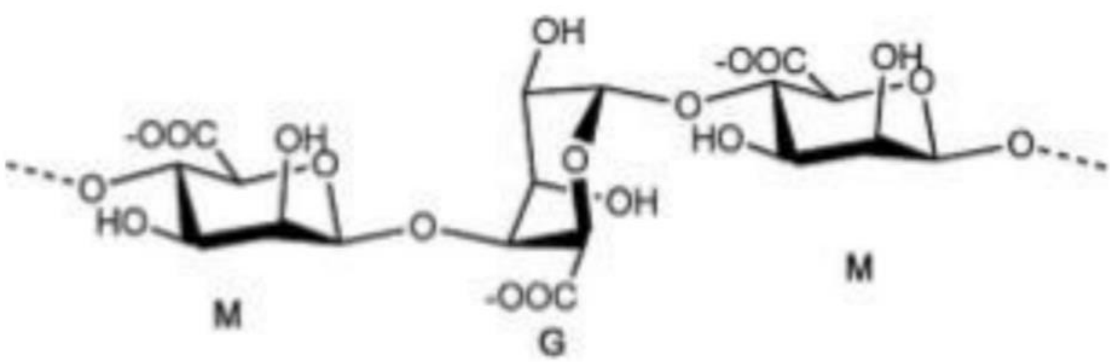


Fig 2. Chemical structure of M-block and G-block in ALG [3]

ALG are composed by β-D-mannuronic acid (M) and α-L-guluronic acid (G), linked together by β-1, 4 and α-1, 4 glycosidic bond respectively. The chemical structure of ALG (Fig.2) contains blocks of (1,4)-linked β-D- mannuronate (M) and α-L-guluronate (G) residues .The blocks can be arranged by consecutive G residues(GGGG),consecutive M residues(MMMM),or by alternative M and G residues(GMGMGM). The G blocks in ALG helps in the crosslinking with divalent cations. The sequence ratio, G block length, molecular weight can influence the hydrogel formation. The increase in G block sequence and molecular weight can increase the mechanical properties of the hydrogels and the physical properties can control the rate of drug delivery [15] and function of encapsulated drug in ALG gels. The ALG extracted from different sources vary in M and G contents in length of the block. SA has lots of hydroxyl groups and less carboxylic acid groups. This provides the opportunity for the formation of intermolecular hydrogen bonding.

Fig 3. SA Powder [16]

1.2 CHARCOAL

Charcoal is an amorphous carbon in the form of highly porous microcrystalline graphite normally obtained from the burning of wood, peat, bones, cellulose or other carbonaceous substance with little or insufficient air. Studies has been revealed the ability of charcoal to adsorb ethylene [17, 18, 19]. Ethylene will react with halogens (chiefly chlorine and bromine) through halogenation and hydro halogenation reactions to form dihaloalkanes. Thus, ethylene can be eliminated from air by passing it over halogenated activated charcoal to form dihalomethane. In many refrigerators activated carbon filter is used to adsorb ethylene gas by cycling the air in it.

Charcoal has been used since earliest times for a large range of purposes including art and medicine [20, 21], but by far its most important use has been as a metallurgical fuel. In industries it was used in large quantities for smelting iron [22]. Prior to industrial revolution, charcoal was occasionally used as a cooking fuel, which causes substantially less air pollution than burning the original uncarbonized organic material wood and certain type charcoal are used for reducing heated metallic oxides to their respective metals:

$$ZnO + C \rightarrow Zn + CO$$

$$Fe_2O_3 + 3C \rightarrow 2Fe + 3CO$$

Fig 4. Charcoal [23]

1.3 HONEY

Honey is a sweet, syrupy, golden coloured natural product from the nectar of flowers by honeybees (*Apis mellifera*). Honey contains mostly sugar, as well as a mix of amino acids, vitamins, minerals, iron, zinc and antioxidants. In addition to its use as a natural sweetener, honey is used as an anti-inflammatory, antioxidant and antibacterial agent. People commonly use honey orally to treat coughs and topically to treat burns and promote wound healing. Next to antibacterial activity, honey exhibits strong antioxidant activity. The phytonutrients in honey are responsible for its antioxidant properties, as well as its antibacterial and antifungal power. They're also thought to be the reason raw honey has shown immune-boosting and anticancer benefits. Above all the honey can act as an efficient plasticizer [24] and the studies were conducted to compare the plasticizing effect of *Apis mellifera* honey on mechanical, physiochemical and optical properties of whey protein isolate films with plasticizing effect of glycerol on whey protein isolated films [25].

Fig 5. Honey [26]

1.4 ASCORBIC ACID

Ascorbic acid (Vitamin C) is a water soluble vitamin in a class of medications called antioxidants found in citrus and other fruits and vegetables, and also available as a dietary supplement. It is an essential nutrient used to prevent and treat scurvy, involved in the formation of collagen, helps wound heal, enhance the absorption of iron, enzymatic production of certain neurotransmitters.

Ascorbic acid and its esters function as antioxidants [27] with some substrates by protecting double bonds and scavenging oxygen. It has been shown to scavenge free radicals directly in the aqueous phases of cells and the circulatory system and has also been proven to protect membrane and other hydrophobic compartments from such damage by regenerating the antioxidant form of vitamin E. Activity of ascorbates has been shown on vegetable oils, animal fats, vitamin A, carotenoids, citrus oils and in fat-containing food such as fish, margarine and milk.

Fig 6. Ascorbic acid tablets [28]

1.5 CITRIC ACID

Citric acid is a colourless organic compound with the chemical formula $HOC(CH_2CO_2H)_2$, found naturally in citrus fruits, especially lemons and limes. It is the most widely used acid in the food industry because it acts as an antioxidant [29], that it protects the body from damaging free radicals and also inhabits the oxidation of other molecules by scavenging reactive oxygen species that are produced as byproducts of normal cellular function. Citric acid is also used to kill harmful bacteria and infections on the skin surface that is common in people with diabetes.

Fig 7. Citric acid [30]

1.6 FOOD PACKAGING

Food packaging plays a major role in protecting and ensuring the quality and safety of food stuffs. It helps to protect food from chemical, biological, environmental and physical contaminants. Generally synthetic packing films are widely used due to some of their desired properties. But total non-biodegradability of such packaging are creating serious ecological issues. To overcome this, an idea of developing bio-degradable polymers for food packaging came to existence. In recent years active packaging technologies are extensively implemented. Active packaging system currently known to date including oxygen scavengers, moisture absorbers, ethylene absorbers, ethanol emitters, time-temperature indicators etc. [31]. Studies were conducted to estimate the efficiency of polymers [32] such as polyesters [33], polycaprolactone, polyaceticacid [34], polyhydroxyalkanoates [35] etc. as packaging films. Such active packaging can extend or maintain the shelf life and quality of foods [36] and these system can be made by coating, incorporating or immobilisation onto the packaging materials [37]. A number of magnificent works are published recently based on innovative active food packaging technologies, natural additives for active food packaging [38], active packaging coatings [37], active edible films [39], packaging concepts for fresh and processed meat [40]. Research works are still in progress in search of efficient packaging materials containing appropriate active agents.

1.7 SCOPE OF THE STUDY

Plastics play a major role in protecting food stuffs in the food supply chain because of their durability cost effectiveness and excellent physical properties. US Environmental Protection Agency reported that above 30% of the municipal solid waste of the year 2005 was generated from packaging related materials [41]. The non-biodegradable nature, chances for leaching of chemicals, issues occurring during plastic recycling process etc. necessitated the development of packaging materials from bio-sourced polymers. Biopolymers such as ALG, cellulose, chitosan etc. are used in recent years as the alternatives for the non-degradable food packaging materials. Among these biopolymers ALG is the most favoured.

ALG is a natural indigestible polysaccharide generally produced by various genera of brown algae [12, 13]. Due to the selective permeability of the polysaccharide film towards oxygen and carbon dioxide gases, it has been used to reduce the respiration of fruits and vegetables [42]. Studies stated that the ALG films can be used to coat fresh cut fruits with high moisture content due to its resistance to being dissolved in water [43]. Ca crosslinked ALG film can be used to pack and preserve meat, fish and poultry products [44, 45, 46, 47]. In a comparative study to prevent the quality loss of cheese by using three different packaging films made up of SA, chitosan, soy protein isolate showed that due to the better wettability of ALG films on products, the ALG coated samples had better qualities [48]. Based on all these studies ALG films can be extensively used in food packaging by incorporating antioxidant, anti browning, anti microbial properties.

2. OBJECTIVES

- Preparation of Ca cross linked ALG films
- Preparation of Charcoal powder
- Preparation of ALG, honey, ascorbic acid, charcoal powder mixed films by solvent casting technique.
- Spectroscopic and microscopic investigation of the developed film.
- Examine the tensile strength and swelling properties of the developed film.
- Examine the surface film wettability of the film.
- Examine the antioxidant property of the film.
- Examine the suitability of the developed film for packaging of fresh cut fruit and vegetable.

3. REVIEW OF LITERATURE

- Valentina Siracusa et al. reviewed different bio-degradable polymers for food packaging applications. Their study provided a detailed discussion about the chemistry behind degradation, mechanical resistance , chemical resistance and barrier properties like oxygen transmission rate, water vapour transmission rate and carbon dioxide transmission rate that a packaging material should posses. They also familiarised applications of some bio-degradable polymers in food packaging field such as polyesters, polycaprolactone, poly lactic acid, polyhydroxyalkanoates, starch based polymers etc. and showed the necessity of more research for the development of this kind of packaging materials [49]

- Carla Vilela et al. reviewed several innovative active agents like oxygen scavengers, primary and secondary antioxidants, antimicrobial agents, carbon dioxide emitters, ethylene scavengers that are used in active food packaging technology for the extension of shelf life of food products by ensuring its safety and quality. It provided basic information about different types of additives for active packaging and its necessity in the current scenario [50].

- Tugce Senturk Parreidt et al. some crucial information about the ALG based edible coatings. They reviewed about the ability of ALG to cross link with divalent metal cations, additives (plasticizers, surfactants, antimicrobials, pigments) that can be incorporated, various methods of film formation (solvent casting, extrusion etc.), ability to preserve (fresh cut fruits, vegetables, meats, poultry, seafood, cheese), moisture barrier applications, gas barrier applications and active compound release applications [51]

- Tugce Senturk Parreidt et al. synthesized uniform SA based edible coating by the ratio of 1.25% SA, 2% glycerol, 0.2% sunflower oil, 1% span 80 (w/w) and studied its water barrier properties using fresh cut cantaloupe and strawberries. Results showed that the film dipped in Ca lactate solution could significantly prevent the water loss in fresh cut cantaloupe but promoted the water loss in strawberry hence suggested further studies to understand the transport mechanism in detail [52]

- Mariana B Osuna et al. developed Ca crosslinked ALG films incorporated with glycerol as plasticizer. The film was loaded with citric acid and ascorbic acid and studied the rate of browning of film due to its degradation and found that the browning of films were only due to the degradation of ascorbic acid and its rate is slowed down in citric acid modified films. So citric acid can be added with ascorbic acid to upgrade its antioxidant property in ALG films and to reduce the browning. This work also permitted the 100% recovery of citric acid and ascorbic acid after casting [27].

- C. R. Badita et al. developed Ca cross linked ALG films with dimensions between 1.43µm and 4.507µm and its characterisation was carried out using different techniques. Chemical interactions of the films were studied using FT-IR spectroscopy which enabled the identification of functional groups and the resultant vibrations for the generation of respective bands. Film morphology was evaluated using SEM and details about the surface structure and roughness were collected with the aid of Atomic Force Microscopy [53].

- Seyhun Gemili et al. synthesized cellulose acetate films and studied the rate of release of low molecular weight natural antioxidants by altering the morphology of the develops films. They changed the cellulose acetate content in the casting solution to alter the pore size of the film and observed that, by the reduction in pore size release of antioxidants like L-ascorbic acid and L-tyrosine is reduced. But reduction in the porosity of the film resulted in its high antioxidant property due to the release of L-ascorbic acid than the L-tyrosine. In conclusion their study revealed that the release of antioxidants can be controlled by changing the pore size and composition of casting solutions [54].

- Robert et al. studied the ability of absorption of activated charcoal with the alternation in its surface area. Activated charcoal with low and high surface area were orally provided for gastrointestinal decontamination. From the results it is understood that the surface area of activated charcoal plays a major role in the absorption of acetaminophen in the gastrointestinal tract because activated charcoal with high surface area increased the absorption by 44% - 85% as compared to the charcoal having low surface area [55].

- Andres Moure et al. studied the antioxidants which can be added in food packaging films to prevent the degradation of vitamins and to preserve the colour and flavour of packed food stuffs. It provided detailed information about the natural sources of antioxidants, antioxidants from residual wastes, its biological activity, potential antioxidant activity and practical applications [10].

- Mehul H. Chudasama reviewed different types of ethylene scavengers that are extensively used in active packaging in recent years. This review is about the role of ethylene in rate of ripening, different types of ethylene scavengers (stochiometric oxidising agents, Potassium permanganate supported adsorbents, activates carbon ethylene removers, nano-halloysite cubes, ethylene scavenging catalysts), effect of ethylene scavenger packaging on shelf life and quality of fruits, vegetables and novel approaches to ethylene removing packagings [17].

4. MATERIALS AND METHODS

4.1 MATERIALS

- SA
- Honey
- $CaCl_2$
- Charcoal powder
- Ascorbic acid
- Citric acid
- Distilled water
- Ethanol
- Acetic acid

4.2 APPARATUS AND GLASSWARES USED

- Glass beakers
- Glass rod
- Measuring jars
- Magnetic stirrer
- Mortar
- Hot air oven
- Homogenizer
- Sonicator
- Teflon pan
- Electronic balance
- Polythene bag

4.3 EXPERIMENTAL METHODS

4.3.1 PREPARATION OF CHARCOAL POWDER

Take some dry, empty coconut shells and introduce them into the stove flame using a holder. If the coconut shell has caught the fire then carefully place the burning coconut shell into a mud container or to a heat proof container and allow them to burn completely. Initially the coconut shell will crack and attain a red colour then changes to black coloured charcoal. Put off the fire by spraying cold water. Wash the obtained charcoal, dry in sunlight and powder it using a mortar.

4.3.2 PREPARATION OF ASCORBIC ACID POWDER

Ascorbic acid tablets are easily available in the medical shop, those tablets are made into fine powder using a mortar.

4.3.3. PREPARATION OF ALG BASED FILMS

3% fixed concentration of the base ALG solution is prepared, 3g (w/v) of SA powder is dissolved in 100ml of distilled water. To this mixture about 6 ml of honey is added and stirred well using a magnetic stirrer, followed by the very slow addition of 20 ml of 1M $CaCl_2$ solution under constant stirring for about 1 hour. Using a homogenizer and a probe sonicator the mixture is homogenized, sonicated, transferred into a preheated teflon pan and dried at 46 ^{0}C for 2 days in a hot air oven. The base film is obtained and is stored in an air tight polythene bag.

4.3.3.1. PREPARATION OF ALG – HONEY- ASCORBIC ACID – $CaCl_2$ FILMS

3g of SA taken in a beaker is dissolved in 100ml distilled water followed by the addition of 6ml honey under constant stirring. To this mixture 1g of ascorbic acid is added and stirring is continued for 5 minutes. Finally 20 ml of 1M $CaCl_2$ solution is added slowly under constant stirring for about 1 hour. The mixture is homogenized, sonicated and then transferred into a preheated teflon pan, dried at 46°C for 2 days in a hot a or oven. The ALG-honey-ascorbic acid film obtained is stored in an air tight polythene bag.

4.3.3.2. PREPARATION OF ALG- HONEY – CITRIC ACID – $CaCl_2$ FILMS

To the mixture of 3g SA in 100 ml of distilled water, 6 ml of honey is added under constant stirring followed by the addition of 1g citric acid. The mixture is cross-linked by the slow addition of 20 ml of 1M $CaCl_2$ solution under constant stirring for 1 hour using a magnetic stirrer. The mixture is homogenized, sonicated and dried in a hot air oven at 46℃ for 2 days after transferring it into a teflon pan. The obtained ALG-honey-citric acid films are stored in an air tight polythene bag.

4.3.3.3. PREPARATION OF ALG – HONEY – CHARCOAL – $CaCl_2$ FILMS

3g of SA powder is dissolved in 100 ml of distilled water. To this mixture 6 ml honey is added and stirred using a magnetic stirrer. 1g of finely powered charcoal is added followed by the addition of 20 ml of $CaCl_2$ solution under constant stirring for about 1 hour. The mixture is then homogenized, sonicated and poured into a preheated teflon pan. The pan is kept in a hot air oven at 52℃ for 2 days to obtain the film and those obtained films are kept in an air tight polythene bag.

4.3.3.4. PREPARATION OF ALG – HONEY – CHARCOAL – ASCORBIC ACID - $CaCl_2$ FILMS

3g of SA is dissolved in 100 ml distilled water and 6 ml honey is added into it under constant stirring. 1g of finely powdered charcoal is added followed by the addition of 1g of powdered ascorbic acid tablets. The solution is cross-linked by the drop wise addition of 20 ml of 1M $CaCl_2$ solution under constant stirring for one hour using a magnetic stirrer. The final mixture is homogenized, sonicated and dried in a hot air oven at 52 ℃ for 2 days by transferring it into a teflon pan. The obtained ALG-honey-charcoal-ascorbic acid films are stored in an air tight polythene bag.

4.3.3.5. PREPARATION OF ALG – HONEY – CHARCOAL – CITRIC ACID – $CaCl_2$ FILMS

To the mixture of 3g SA in 100 ml distilled water, 6 ml honey is added under constant stirring followed by the addition of 1g finely powdered charcoal. This mixture is treated with 1g of citric acid and cross linked by 20 ml of 1M $CaCl_2$ solution under constant stirring for about 1 hour using a magnetic stirrer. The mixture is homogenized, sonicated and dried in a hot air oven at 52°C for 2 days after pouring it in a teflon pan. The developed ALG-honey-charcoal-citric acid films are stored in an air tight polythene bag.

4.3.4. FILM CHARACTERISATION

4.3.4.1. SURFACE FILM WETTABILITY

Surface wettability, gives the information about the interaction of water with a surface, which plays a crucial role in almost all biological, physical and chemical processes. The value of contact angle indicates how the water can wet the surface of the sample to be analysed. Low value of contact angle shows the surface tendency to spread the water on its surface and high value indicates the tendency of surface to repel the water. The measurements can be taken by direct method Sessile-drop goniometry. The static contact angle measurements were performed through the equipment OCA 20 (Data Physics) using water (10 µl of volume drop and 2 µl/s of velocity). For each condition, ten measurements were performed using the Sessile-drop method [03].

4.3.4.2. SEM ANALYSIS

SEM analysis is mainly for surface morphological and topographical studies and also for compositional elemental analysis. The morphology of the developed films were examined using Carl Zeiss EVO 18 Research which provides excellent quality imaging results from an analytical microscope. The image is generated by scanning the sample with a focused beam of electrons. Interaction of electrons with sample surface creates various signals that contains information about the surface topography and composition of the samples. The detected signal is analysed to produce an image of the sample surface**.**

4.3.4.3. TENSILE STRENGTH

Mechanical properties of the developed films can be detected by tensile tests and can compare with existing similar films to examine its efficiency. Tests can be done both in dry and wet states and performed according to ASTM D882 standard tensile testing procedure. To get accurate information about the extend of breakage on stretch the results were evaluated in terms of tensile strength. Tensile strength can be determined by the equation,

$$\mathbf{TS = F_{max} / A} \qquad \textbf{(1)}$$

where, F_{max} indicates the maximum force at break. A represents the cross sectional area of the specimen [3].

4.3.4.4. DPPH RADICAL SCAVENGING ASSAY

Radical scavenging activity of the test sample against stable 2, 2- diphenyl 2-picrylhydrazyl hydrate (DPPH) was determined according to the method of Brand-William et al., (1995) with slight modification [56] . DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at the optical density 515 nm on a UV visible spectrophotometer. For DPPH assay the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ ml; w/v). A 60µM solution of DPPH in methanol was freshly prepared and a 200µl of this solution was mixed with 50µl of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50,100,200,400,800 µg/ml). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank. Radical scavenging activity was calculated by the following formula,

$$\boldsymbol{Percentage\ inhibition} = \frac{\textbf{absorbance of control} - \textbf{absrobance of test}}{\textbf{absorbance of control}}\ \boldsymbol{X\ 100} \qquad \textbf{(2)}$$

4.3.4.5. OPTICAL IMAGE

Optical image of the film is generated using Olympus BX61 Upright Optical Microscope which is used in life sciences and cell biology for phase contrast, brightfield, darkfield, differential interference contrast (DIC), polarization, or fluorescence microscopy of slide samples. Upright microscopes can also be used in the microscopy of fixed cells or tissue samples.

4.3.4.6. FT-IR ANALYSIS

The chemical composition of the raw materials (ALG, charcoal, ascorbic acid etc.) and their possible interactions can be identified using Infrared Spectroscopy. FT-IR spectra were on a Perkin Elmer FT-IR Spectrometer, which is used to find out the important functional groups present in the molecule.

4.3.4.7. BIODEGRADATION ANALYSIS

For biodegradation analysis, the samples were cut into (2cm × 2 cm), dried in an air oven at 100°C and weighed (W1). The films were then buried in plastic boxes at a depth of 10 cm from the soil to ensure aerobic degradation. After stipulated period, the samples were taken out from the soil, washed to remove soil particles and dried in the oven at 105°C and weighed. The extent of degradation was measured in terms of % weight loss using the following equation,

$$\mathbf{WL}(\%) = \frac{\mathbf{W1 - W2}}{\mathbf{W1}} \, \boldsymbol{X}\,\mathbf{100} \qquad \textbf{(3)}$$

4.3.4.8. EXAMINATION OF SUITABILITY OF THE DEVELOPED FILMS

4.3.4.8.1 EXAMINATION OF OXIDATION RATE OF FRESH CUT FRUITS AND VEGETABLES

Rate of oxidation of fresh cut potato and apple wrapped with and without the developed films are examined by visual detection. The control and the film coated samples are kept at room temperature and the changes are observed.

4.3.4.8.2 EXAMINATION OF RIPENING RATE OF FRESH CUT FRUITS AND VEGETABLES

A partially ripened fruit (a variety of banana), is taken, one is wrapped with the synthesised film and the other is taken as the control. Both are kept at room temperature and the ripening rate of these two samples are noted by visual detection.

5. RESULTS AND DISCUSSION

Due to the easy availability, bio-compatibility, low cost and its high blending property ALG is chosen as the matrix for the film preparation. The base film is prepared by stirring the honey added ALG solution using a magnetic stirrer followed by the drop wise addition of CaCl2 for 1 hour as cross linking agent. This final mixture is converted into the film by placing it in a vacuum air oven for 2 days at 46°C. Similarly materials like ascorbic acid, citric acid and charcoal are incorporated in the base film to obtain different films for active food packaging which can alter the oxidation, browning and ripening of fresh cut fruits and vegetables.

5.1. ALG FILMS

5.1.1. ALG-HONEY-$CaCl_2$ FILMS

ALG solution containing 6 ml honey is cross linked using CaCl2 solution by continuously stirring by a magnetic stirrer and is homogenized and sonicated. This mixture is transferred into a preheated teflon pan and placed in a vacuum air oven at 46°C for 2 days to obtain a transparent, thin, homogeneous, smooth ALG-honey-CaCl2 film.

Fig 8. ALG –Honey–$CaCl_2$ Film

5.1.2. ALG-HONEY-ASCORBIC ACID-$CaCl_2$ FILMS

ALG solution containing 6 ml honey is continuously stirred using a magnetic stirrer followed by the addition of 1g finely powdered ascorbic acid tablets and the solution is cross linked by the drop wise addition of 1M CaCl2 solution for about 1 hour. Thus obtained mixture is homogenized, sonicated and placed in a vacuum air oven by transferring the solution in a preheated teflon pan for 2 days at 46°C to obtain a homogeneous, dry and slightly whitish ALG-honey-ascorbic acid-CaCl2 film.

Fig 9. ALG-Honey-Ascorbic acid –$CaCl_2$ Film

5.1.3. **ALG-HONEY-CITRIC ACID-CaCl2 FILMS**

ALG solution containing 6 ml honey is continuously stirred using a magnetic stirrer followed by the addition of 1g finely powdered citric acid crystals and the solution is cross linked by the drop wise addition of 1M $CaCl_2$ solution for about 1 hour. Thus obtained mixture is homogenized, sonicated and placed in a vacuum air oven by transferring the solution in a preheated teflon pan for 2 days at 46°C to obtain a homogeneous, smooth, partially transparent and brown coloured ALG-honey-citric acid-$CaCl_2$ film.

Fig 10. ALG-Honey-Citric acid-$CaCl_2$ Film

5.1.4. ALG-HONEY-CHARCOAL-$CaCl_2$ FILMS

ALG solution containing 6 ml honey is continuously stirred using a magnetic stirrer followed by the addition of 1g finely powdered charcoal and the solution is cross linked by the drop wise addition of 1M $CaCl_2$ solution for about 1 hour. Thus obtained mixture is homogenized, sonicated and placed in a vacuum air oven by transferring the solution in a preheated teflon pan for 2 days at 52°C to obtain a homogeneous, smooth, opaque and black coloured ALG-honey-charcoal-$CaCl_2$ film.

Fig 11. ALG-Honey-Charcoal-$CaCl_2$ film

5.1.5. ALG-HONEY-CHARCOAL-ASCORBIC ACID FILMS

ALG solution containing 6 ml honey is continuously stirred using a magnetic stirrer followed by the addition of 1g finely powdered charcoal and ascorbic acid tablets. The solution is cross linked by the drop wise addition of 1M $CaCl_2$ solution for about 1 hour. Thus obtained mixture is homogenized, sonicated and placed in a vacuum air oven by transferring the solution in a preheated teflon pan for 2 days at 52°C to obtain a homogeneous, rough, opaque and black coloured ALG-honey-charcoal-ascorbic acid-$CaCl_2$ film.

Fig 12. ALG-Honey-Charcoal-Ascorbic acid-CaCl2 Film

5.1.6 ALG-HONEY-CHARCOAL-CITRIC ACID-$CaCl_2$ FILMS

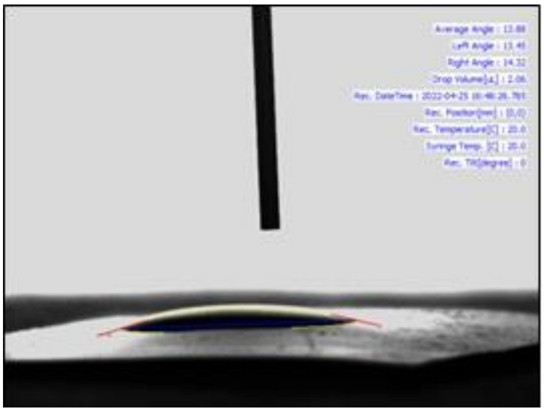
ALG solution containing 6 ml honey is continuously stirred using a magnetic stirrer followed by the addition of 1g finely powdered charcoal and citric acid crystals. The solution is cross linked by the drop wise addition of 1M $CaCl_2$ solution for about 1 hour. Thus obtained mixture is homogenized, sonicated and placed in a vacuum air oven by transferring the solution in a preheated teflon pan for 2 days at 52°C to obtain a homogeneous, smooth, opaque and black coloured ALG-honey-charcoal-citric acid-$CaCl_2$ film.

Fig 13. ALG-Honey-Charcoal-Citric acid-$CaCl_2$ film

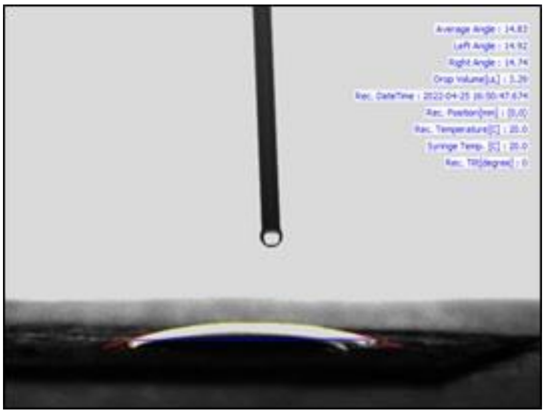
5.2. FILM CHARACTERISATION

5.2.1. SURFACE FILM WETTABILITY

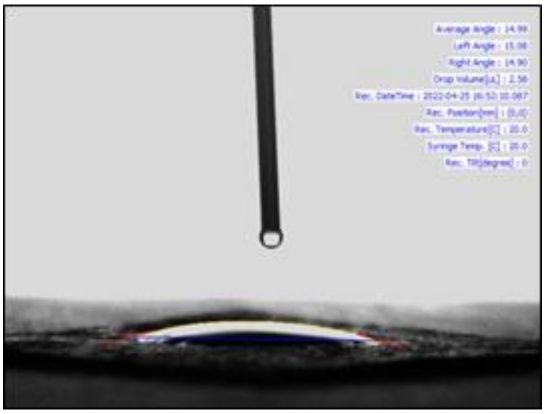
Contact angle measurement is carried out to determine the ability of a liquid to wet the surface of a solid. In water contact angle measurement a water drop comes in contact with the surface of the sample to be analysed, an angle is formed by the water at the three-phase boundary where a liquid - gas and solid intersects. Based on the value on angle made by the water droplet on the solid, surface of a material can be classified as super wetting ($\theta \approx 0°$), super hydrophilic(θ<10°), hydrophilic(10°<θ<90°), hydrophobic(90°<θ<150) and super hydrophobic (θ>150°). The results showed that the base ALG film, charcoal modified ALG film and citric acid modified ALG film are hydrophilic in nature with an average contact angle of 13.88°, 14.83° and 14.99° respectively. The slight increment in the water contact angle is shown by the addition of charcoal and citric acid in the base film but this small change in the value didn't make any drastic change in the film's hydrophilicity, hence it can be used to retain the freshness of fruits while transporting and storing. As a result of anaerobic respiration all plant tissues produces ethanol which plays a significant role in the ripening of fruits [57]. The hydrophilic nature of SA can be exploited to embed molybdenum disulfide nano sheet for ethanol dehydration and thus can separate the formed ethanol content [58]. The hydrophilic nature also results in the oxygen barrier properties at different relative humidities, water vapour barrier property and low humidity for the packaging films.



(a)



(b)



(c)

Fig 14. Contact angle measurements of (a) Base ALG film, (b) ALG film Modified with Charcoal (c) ALG film Modified with Charcoal and Citric acid

Sample	**Contact Angle (°)**
Base ALG Film	13.88
ALG + Charcoal	14.83
ALG + Charcoal +Citric acid	14.99

Table 1. Contact Angle measurements

5.2.2. SEM ANALYSIS

SEM analysis provides information about the surface morphology, structural variation, particle size and its distribution on the sample surface. High resolution images are obtained by striking an electron beam on to the sample surface followed by the analysis of the diffracted electrons. Here the surface morphology of the ALG-honey-charcoal-ascorbic acid-$CaCl_2$ film is observed under SEM analysis. Fig.15 represents the SEM image of the film. From the image it is clear that the developed film is highly porous in nature and the charcoal powder and ascorbic acid are well distributed in the whole matrix.

Highly porous materials show a wide range of physical, thermal, mechanical and electrical properties due to their large specific surface area. As the porosity increases surface area of a material also increases [54]. Due to the increased area film can effectively interact with the food stuffs wrapped in it and thereby shows high antioxidant potential and ethylene scavenging property. Thus we can significantly reduce the browning of fresh cut products arises due to oxidation and prolong its storage. Such films can also increase the shelf life of partially ripened fruits by effectively scavenging the ethylene released, due to the high absorptivity of film as a result of increased specific surface area [55].

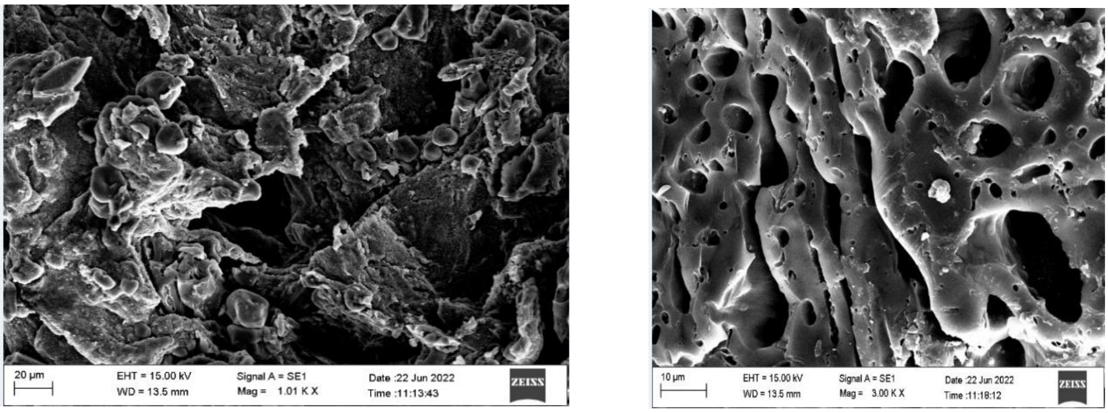


Fig 15. SEM image of the ALG-Honey-Ascorbic acid-$CaCl_2$ Film

5.2.3. TENSILE STRENGTH

Mechanical characteristics of a food packaging material plays a major role in its application. From the study of bio-degradable plastics from cassava peels by adding stem powder conducted by M. Muhaimin and his coworkers, it is understood that the tensile strength of the developed film is in the range of 1.5 - 2.9 MPa [59]. Starch films usually possess a very low tensile strength of 0.35 - 1.5 MPa [60].The average tensile strength of the developed film is obtained as 1.95 MPa at an average maximum load of 24.6142 N which is comparable with the tensile strength of existing bio-degradable plastic films.

Even though the natural plastics didn't have the tensile strength similar to conventional plastics but its strength can be increased by the addition of suitable substrates. Research works are still in progress to enhance the tensile strength of bio-degradable films.

	Thickness (mm)	Tensile strength (MPa)	Maximum load (N)	Load at Break (N)	Elongation at Break (%)
1	0.35	2.43	29.7702	-2.04	23.36
2	0.40	1.19	16.7108	0.30	29.23
3	0.35	2.23	27.3615	-0.59	18.11
Mean	0.37	1.95	24.6142	-0.78	23.57
Std: deviation	0.03	0.66	6.95	1.18	5.56
Coeff: of variation	7.87	34.03	28.23	-152.22	23.60

Table 2. Tensile strength of ALG-Honey-Ascorbic acid-$CaCl_2$ film

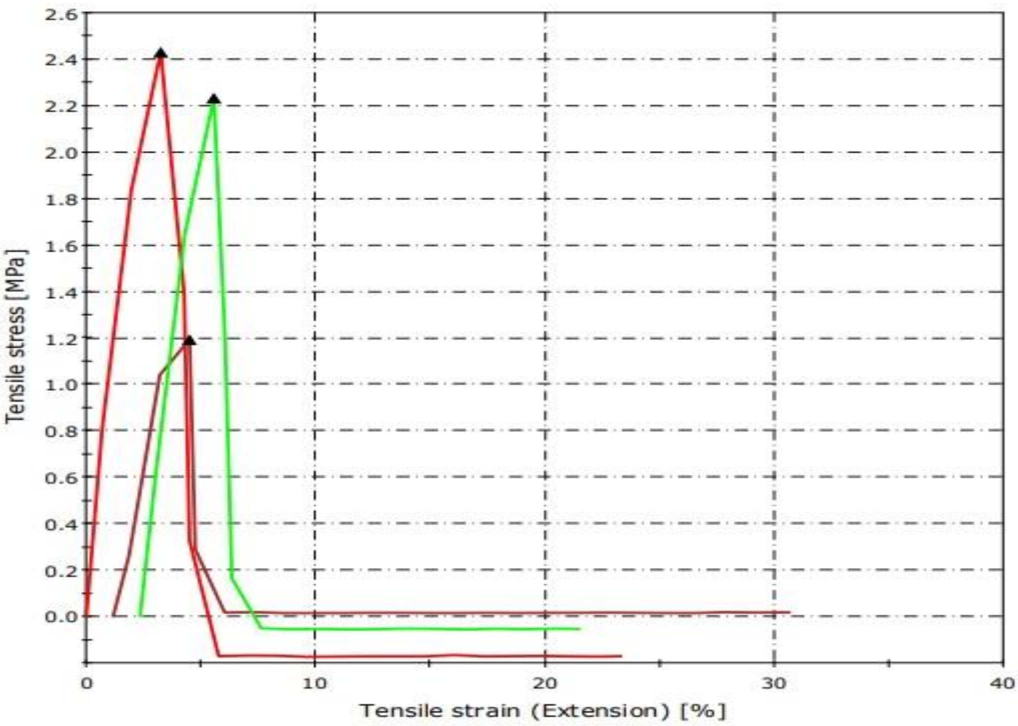


Fig 16. Graphical representation of tensile strength

5.2.4. DPPH ASSAY

In DPPH Assay radical scavenging activity of the test sample against stable 2, 2- diphenyl 2-picrylhydrazyl hydrate was determined. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH resulting a change in colour from deep violet to light yellow and the absorbance was measured at 515 nm. For high antioxidant property the assay will yield a sudden colour change. IC50 value indicates the amount of sample needed to inhibit the oxidation process by half.

DPPH radical scavenging assay of solution containing ALG-honey-citric acid-charcoal-$CaCl_2$ and ALG-honey-ascorbic acid-charcoal-$CaCl_2$ were conducted and the citric acid incorporated solution didn't show a noticeable antioxidant property but ascorbic acid incorporated solution showed a very high antioxidant property of IC50 value less than 1.56 µg/ml as compared to the standard. In case of the standard the colour change from violet to light yellow due to the reduction of DPPH is observed only by increasing the concentration of standard but in case of ascorbic acid sample the colour change is observed at the minimum concentration which indicates the high potential of the film to exhibit antioxidant property. Hence it is evident that the developed film can act as a better antioxidant packaging for fresh cut fruits and vegetable and there by reduces its browning and increases shelf life.

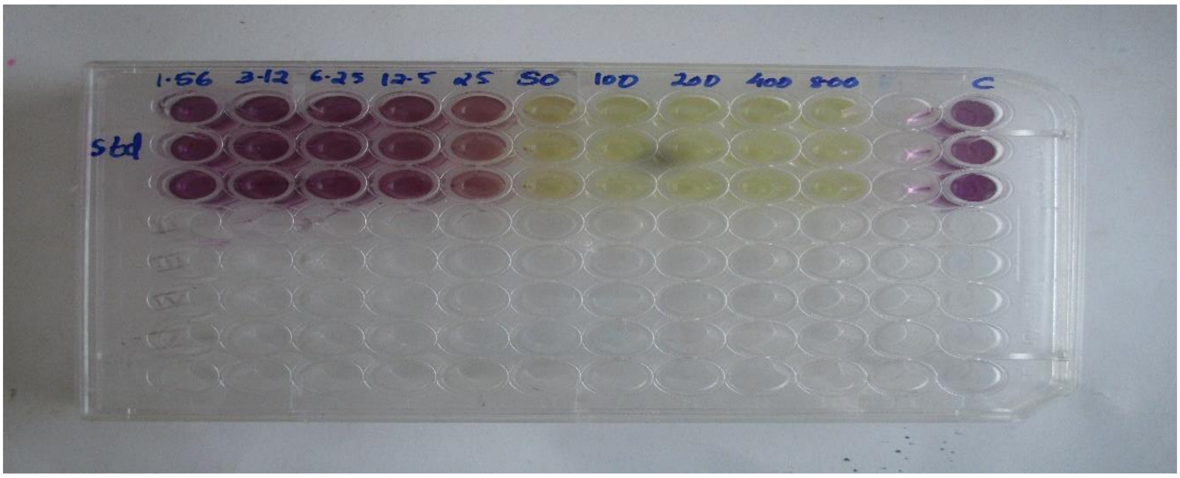


Fig 17.DPPH assay of Standard

Standard	Concentration (µg/ml)	OD at 515nm			Avg. OD at 515nm	% of Inhibition
Control	-	1.28	1.29	1.27	1.28	-
Ascorbic acid (standard)	1.56	1.24	1.17	1.20	1.20	6.0
	3.12	1.23	1.20	1.15	1.19	6.8
	6.25	1.13	1.10	1.14	1.12	12.2
	12.5	0.98	0.93	0.97	0.96	25.0
	25	0.73	0.57	0.67	0.65	48.7
	50	0.09	0.09	0.09	0.09	93.0
	100	0.09	0.08	0.09	0.09	93.2
	200	0.08	0.09	0.08	0.08	93.5
	400	0.09	0.08	0.09	0.08	93.2
	800	0.09	0.08	0.09	0.09	93.2
IC 50	**26.29**					

Table 3. DPPH Assay of Standard

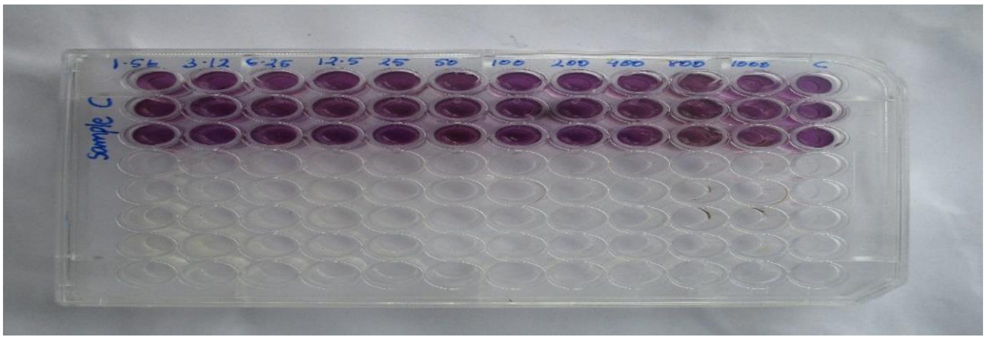


Fig 18. DPPH Assay of Citric acid incorporated solution

Standard	Concentration (µg/ml)	OD at 515nm			Avg. OD at 515nm	% of Inhibition
Control	-	0.8866	0.8861	0.8863	0.8863	-
Citric acid	1.56	0.8666	0.8668	0.8669	0.8668	2.20
	3.12	0.8282	0.8292	0.8288	0.8287	6.50
	6.25	0.7841	0.7845	0.7849	0.7845	11.49
	12.5	0.7750	0.7754	0.7755	0.7753	12.58
	25	0.7252	0.7253	0.7252	0.7253	18.17
	50	0.7118	0.7113	0.7115	0.7115	19.72
	100	0.6884	0.6886	0.6889	0.6886	22.30
	200	0.6661	0.6661	0.6668	0.6663	24.82
	400	0.6423	0.6425	0.6435	0.6428	27.48
	800	0.6111	0.6111	0.6116	0.6113	31.03
IC 50	-					

Table 4. DPPH Assay of Citric acid incorporated solution

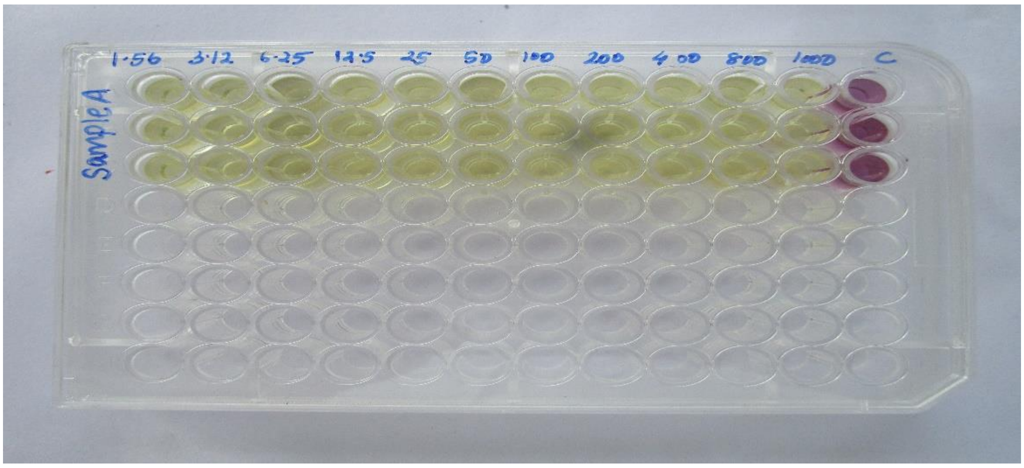


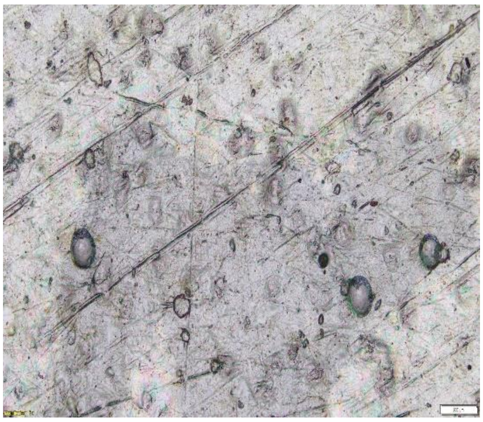
Fig 19. DPPH Assay of Ascorbic acid incorporated solution

Sample code	Concentration (µg/ml)	OD1	OD2	OD3	Avg. OD at 515nm	% of Inhibition
Control	-	0.8886	0.8880	0.8881	0.8882	
Ascorbic acid	1.56	0.3083	0.3085	0.3084	0.3084	65.28
	3.12	0.2983	0.2982	0.2982	0.2982	66.42
	6.25	0.2714	0.2711	0.2712	0.2712	69.46
	12.5	0.2674	0.2670	0.2673	0.2672	69.91
	25	0.2537	0.2540	0.2535	0.2537	71.43
	50	0.2394	0.2397	0.2396	0.2396	73.03
	100	0.2085	0.2087	0.2082	0.2085	76.53
	200	0.1933	0.1938	0.1937	0.1936	78.20
	400	0.1803	0.1802	0.1808	0.1804	79.69
	800	0.1557	0.1554	0.1558	0.1556	82.48
	1000	0.1330	0.1332	0.1333	0.1332	85.01
	IC50 value	**<1.56 µg/ml**				

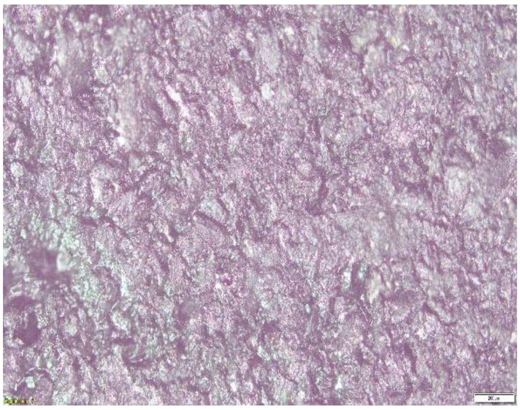
Table 5. DPPH Assay of Ascorbic acid incorporated solution

5.2.5. OPTICAL IMAGE

By taking optical images of the developed films, the microscopical structures of the films were understood. Base film exhibited a plane, homogeneous structure and its roughness increased by the subsequent addition of charcoal, citric acid and ascorbic acid powders. The most rough surface was examined in the ALG-honey-ascorbic acid-$CaCl_2$ films. The optical images of different films are illustrated below.



(a)



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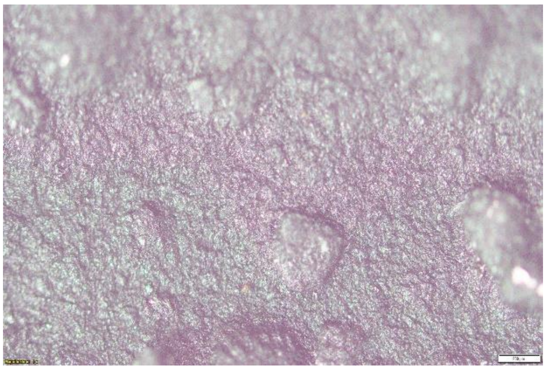
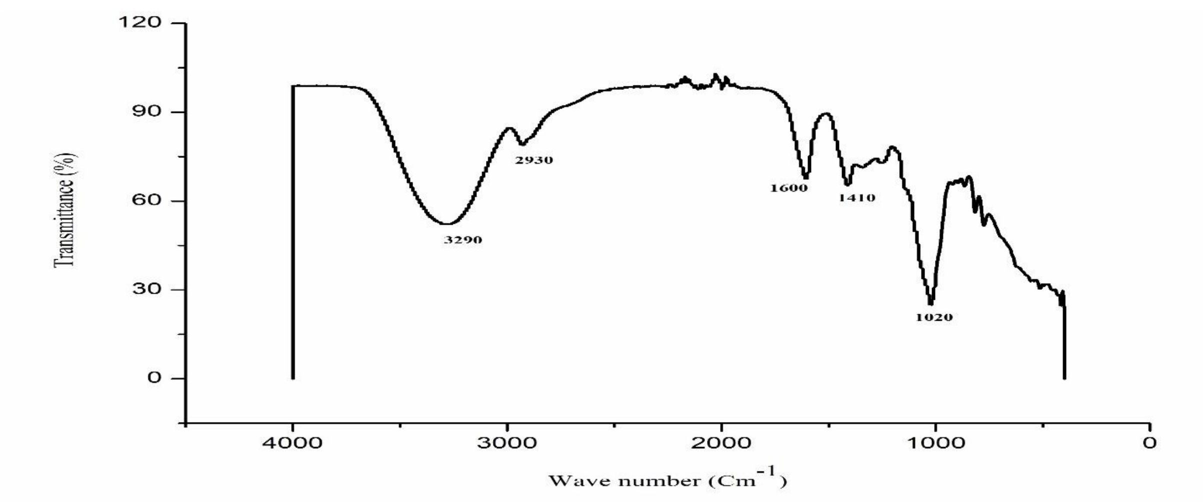


(c)

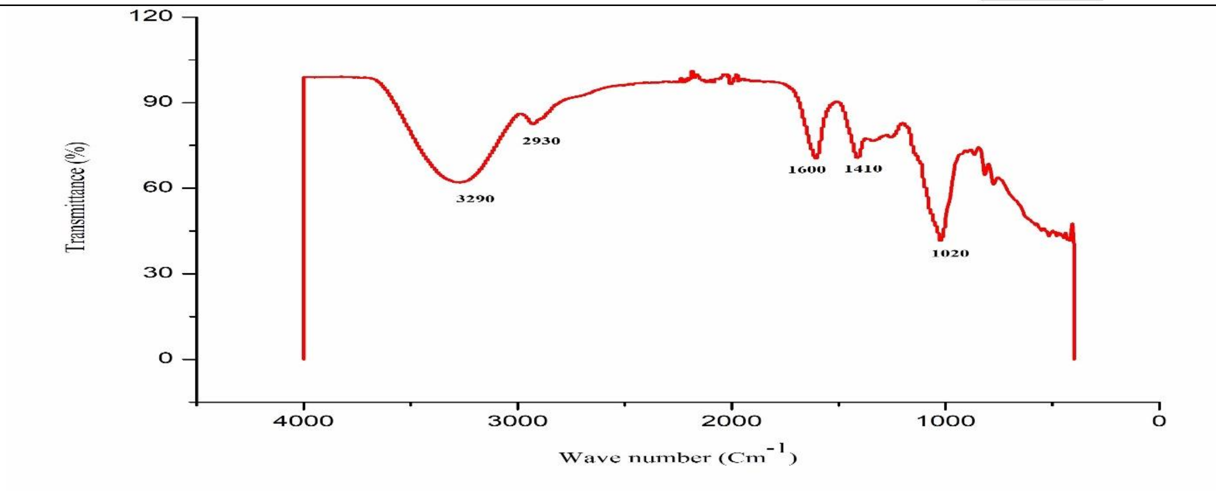
(d)

Fig 20. Optical image of (a) Base ALG film, (b) ALG film Modified with Charcoal (c) ALG film Modified with Charcoal and Citric acid   (d) ALG film Modified with Charcoal and Ascorbic acid

5.2.6. FT-IR ANALYSIS



(a)



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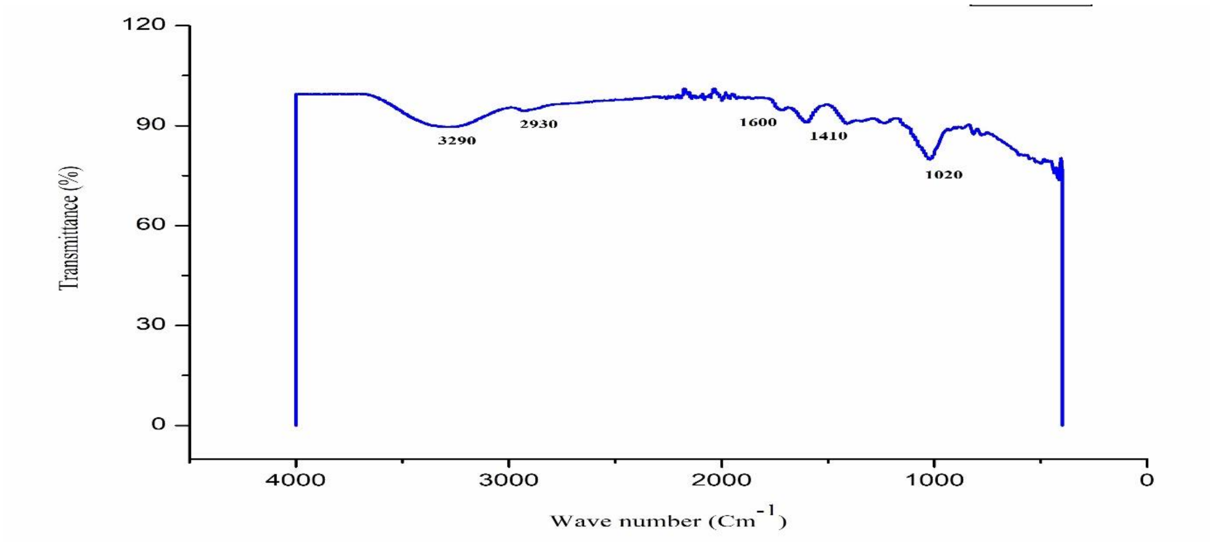
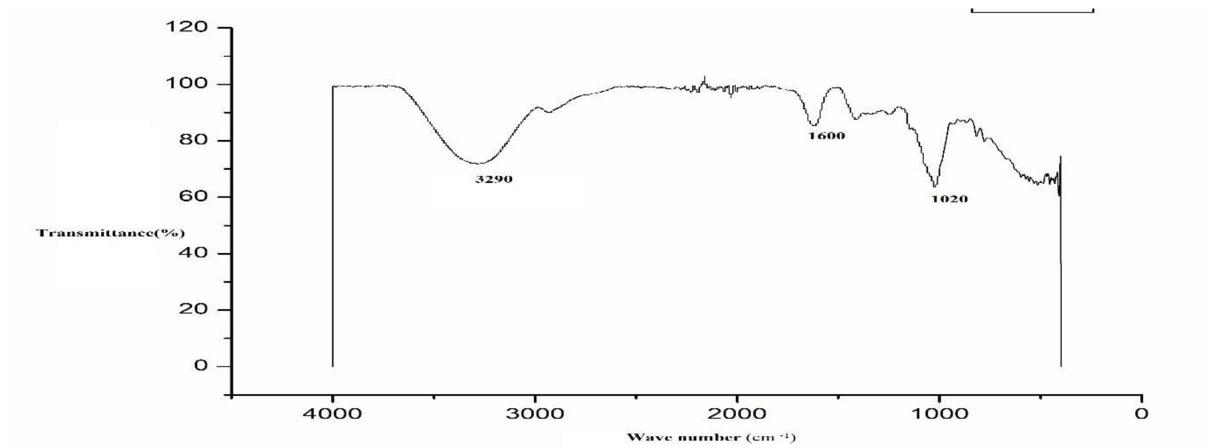


(c)



(d)

Fig 21. FT-IR Spectra of (a) Base ALG film, (b) ALG film Modified with Charcoal (c) ALG film Modified with Charcoal and Citric acid (d) ALG film Modified with Charcoal and Ascorbic acid

The large absorption band in the FTIR spectrum of SA in the range of 3600-3000 cm^{-1} indicates the stretching vibration bands of OH group and peak at 2930 cm depicts the CH vibration. The observed bands around 1600 cm^{-1} and 1410 cm^{-1} indicates the asymmetric and symmetric stretching vibrations of the COO^- groups which are specific to ionic bonding. A higher intensity peak at 1020 cm^{-1} represents the strong binding of Ca^{2+} to SA or stronger OH binding vibration [53].

The FT-IR spectrum of charcoal exhibited primarily 3 broad bands around 3450, 1640 and 1400 cm^{-1} representing the OH stretching, C=O/C=C stretching and carboxylate stretching respectively [61], hence the percentage of transmittance or the peak intensity varies from the FT-IR peak of the base film.

It was found that citric acid showed the broadening of OH stretching around 3920 cm^{-1}, C=O stretching near 1700 cm^{-1} and C-OH stretching near 1100 cm^{-1}[62].

The spectrum of ascorbic acid revealed that the stretching vibration of C=C and peak of enol-hydroxyl observed around 1670 cm^{-1} and 1320 cm^{-1} respectively [63].

5.2.7. BIO-DEGRADATION ANALYSIS

From a single day observation ALG films showed an average degradation of 23.38% which becomes a great advantage over the existing non-biodegradable food packaging materials. High bio-degradability results in the sustainable disposal of the packaging material and will not cause a minimum level of harmful effects as compared to the disposal of conventional packaging materials. The rate of bio-degradability is illustrated below.

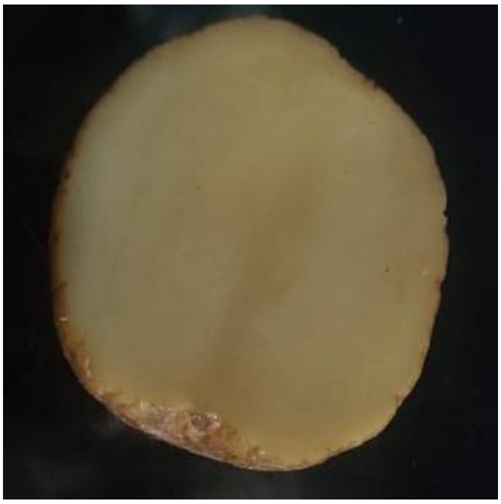
FILM	**INITIAL WEIGHT (g)**	**FINAL WEIGHT (g)**	**WEIGHT LOSS (%)**
ALG	0.2049	0.1600	21.91
ALG + Charcoal	0.1671	0.1379	21.17
ALG + Charcoal + Citric acid	0.2882	0.1862	35.39
ALG + Charcoal + Ascorbic acid	0.2894	0.2458	15.06

Table 6. Rate of Bio-degradability

5.2.8. EXAMINATION OF SUITABILITY OF THE DEVELOPED FILMS

In the comparative study of examining the oxidation of film wrapped fresh cut potato and apple with its control showed a significant difference in their appearance within 24 hours. The rate of browning of fresh cut products due to oxidation is slower in citric acid, slowest in ascorbic acid in comparison with the control which indicates the application of the developed films as good antioxidant films for the packaging of fresh cut products.

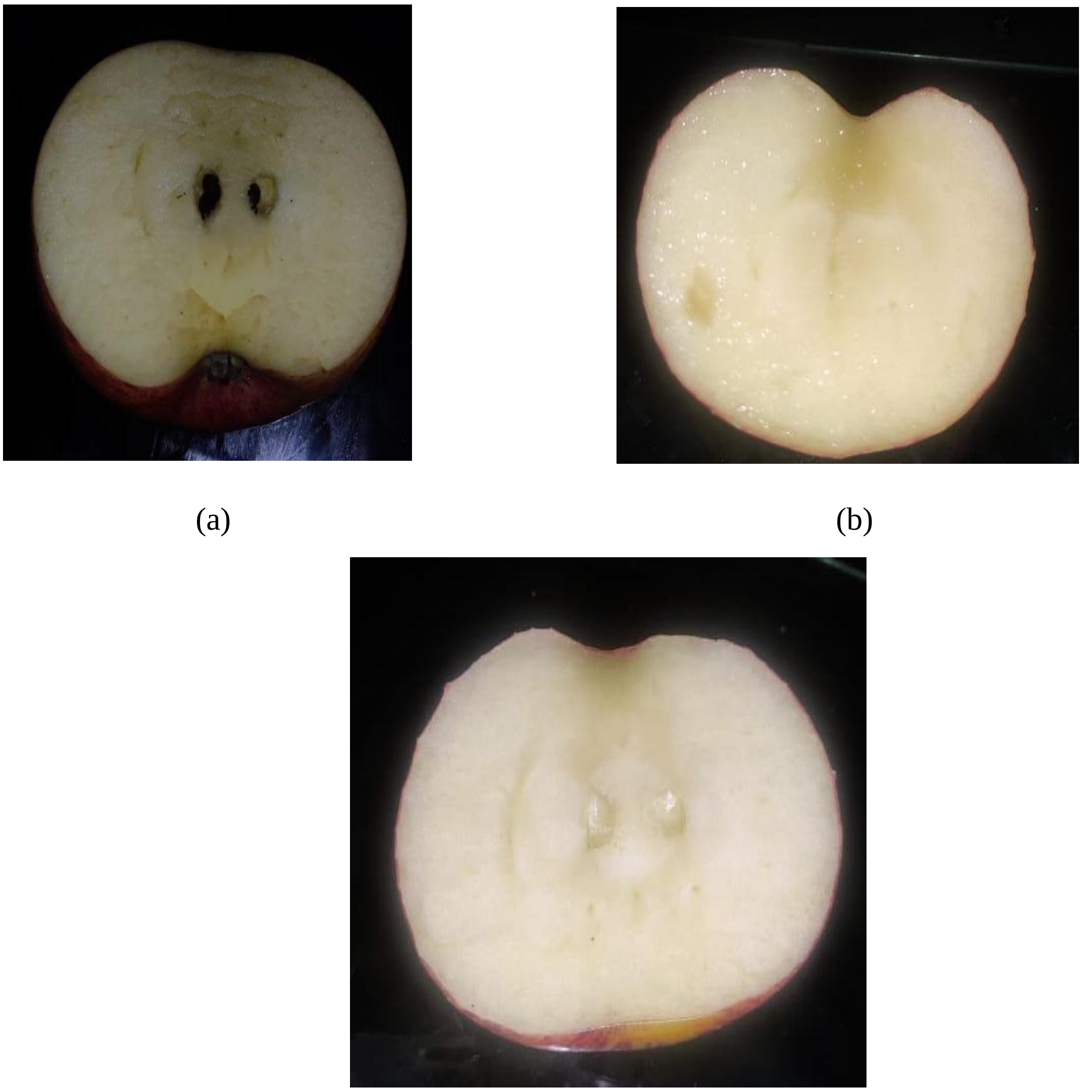
(a)



(b)

(c)

Fig 22. Browning of fresh cut potato due to oxidation in (a) control (b) potato wrapped with Citric acid modified film (c) potato wrapped with Ascorbic acid modified film



(a) (b)

(c)

Fig 23. Browning of fresh cut apple due to oxidation in (a) control (b) apple wrapped with Citric acid modified film (c) apple wrapped with Ascorbic acid modified film

The ripening rate of banana wrapped in the developed charcoal film is also compared with the control and observed a difference on their ripening rate. The control get ripened fast and started to decay within 5 days and the banana wrapped in the film undergone a colour change on its peel but didn't undergo decay which makes the film suitable for packaging of partially ripened fruits during transportation and storage.

(a_1)

(a_2)

(b_1)

(b_2)

(c_1)

(c_2)

Fig 24. Comparison of ripening of Banana wrapped in Charcoal film with control

(a_1) banana at first day, (b_1) banana wrapped in charcoal film after two days, (c_1) banana wrapped in charcoal film after five days , (a_2) control at first day, (b_2) control after two days, (c_2) control after five days

6. CONCLUSION

For the packaging of food stuffs plastics are the desirable material due to their extraordinary properties. But problems associated with its handling necessitated the development of novel bio sourced plastics. Better film forming property of ALG makes the ALG modified films a major part in the research of food packaging materials. Active food packaging using natural materials are acquiring great intentness in the current scenario. In this study films made up of ALG, ascorbic acid, citric acid, charcoal and honey were prepared successfully by solvent casting technique. From DPPH assay it is clear that the addition of ascorbic acid increased the antioxidant property of the film to a great extend so that the film can effectively reduce the oxidation of fresh cut fruits and vegetable and browning by increasing its shelf life. By examining the suitability of the developed film we could observe that the film also posses an ethylene scavenging property so can prevent the fast ripening and reduce the wastage of fruits and vegetables. Uniform distribution of charcoal, ascorbic acid and highly porous nature of the film surface is identified using SEM analysis which also enhances the anti-oxidant and ethylene scavenging property of the film due to its increased specific surface area. FTIR spectrum helped to understand the functional groups within the film and developed film exhibited high bio-degradable property which can never be attained by the conventional plastics. Even though the developed film exhibited a tensile strength within the range of currently exiting bio-degradable plastics, it can't compete with the strength of plastics which is usually greater than 30 MPa. Research works are still in progress to enhance the tensile strength of bio-degradable films. Continuous research and developments are required to take complete advantage of biodegradable plastics for socio-economic benefits because there is no doubt that both bio-sourced and bio-degradable plastics can create a revolutionary change in the future.

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