PREPERATION AND FUNCTIONAL CHARACTERISATION OF BIOPOLYMER (ARABINOXYLAN AND CELLULOSE) BASED FILMS/COATINGS

A

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CERTIFICATE

This is to certify that **Ms. Mannat Aneja** completed her research project entitled **"Preparation and functional characterization of biopolymer (Arabinoxylan and Cellulose) based films/coatings"**. This thesis is submitted to Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala in the partial fulfilment of the requirement for the award of the degree of Masters of Science in Biotechnology. She joined National Food Agri-Food Biotechnology Institute (NABI) on 8th January, 2019 for her project work.

Koushik Mazumder.

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Certificate

This is to certify that the thesis entitled **PREPERATION AND FUNCTIONAL CHARACTERISATION OF BIOPOLYMER (ARABINOXYLAN AND CELLULOSE) BASED FILMS/COATINGS** being submitted by **Ms. Mannat Aneja (Roll no: 301701017)** in partial fulfilment of the requirements for the award of degree of Masters of Science in Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab is a bonafide work carried out under the supervision and conception of Dr. Niranjan Das and no part of this thesis has been submitted for the award of any degree.

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Declaration

I hereby declare that the project report entitled "**PREPERATION AND FUNCTIONAL CHARACTERISATION OF BIOPOLYMER (ARABINOXYLAN AND CELLULOSE) BASED FILMS/COATINGS**" submitted by me to THAPAR INSTITUTE OF ENGINEERING AND TECHNOLOGY, PATIALA in partial fulfillment of the requirement for the award of degree of MASTER IN SCIENCE in BIOTECHNOLOGY is a record of project work carried out by me under the guidance of Dr. Koushik Mazumder in NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE, (NABI) MOHALI. I further declare that this is the submission of my own work and that to the best of my knowledge and belief, it contains no material previously published or written by any other person or institution.

Mannat Aneja.

Signature

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Date: 16/8/19

place: Patiala

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LIST OF ABBREVIATIONS

mg - milligram µg -

microgram g - gram ^{0}C -

degree Celsius µl - micro

litre M - molar rpm -

revolution per minute

% - percentage µm

- micro meter

AX - arabinoxylan

AA - acetic acid

H_2SO_4 - sulphuric acid

$NaBH_4$ - sodium borohydride

NaOH- sodium hydroxide

HCl - hydrochloric acid

SAC- Stearic acid-cellulose esters

LAC- Lauric acid-cellulose esters

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ABSTRACT

Vast amounts of by-products are generated every year from agricultural crop production and hence great quantities of polysaccharides remain underutilized. The polysaccharides from agricultural by-product can be separated and used in the form of new materials. This project devoted to the possibility of using hemicelluloses for special polysaccharide film applications in the packaging sector, starting from hemicellulose isolations from a side product of agricultural processes, hemicellulose characterization and assessing material properties and the potential use of hemicellulose films in later applications. First, Arabinoxylan and cellulose were extracted from wheat straw. After that, cellulose was esterified by using alkali. Films were prepared by mixing arabinoxylan with fatty acid esterified cellulose. In order to characterize the esterified cellulose, FTIR of the sample was carried out. The presence of carbohydrate within the film was also estimated by phenol sulphuric method. The compositional analysis of film was done using gas chromatography mass spectrophotometery (GC-MS). Furthermore, in order to understand the structural characterization, scanning electron microscopy (SEM), water vapor permeability rate (WVPR) and the color of films were analysed.

Keywords :- Wheat straw, Arabinoxylan, Cellulose, Cellulose fatty acid esters, FTIR, GC-MS, SEM, WVPR, Color.

INTRODUCTION

The issue of food losses is of high and global importance in the efforts to combat hunger, raise income and improve food security in countries of the world. Food losses imposes the impact on food safety and quality, food security for poor people, on economical growth and environment.

Food losses are maximum after the harvesting. Fruits and vegetables are highly perishable in nature and about 25-30% of post harvest losses in fruits and vegetables are due to absence of post harvest treatments, traditional storage on farms and infestation of microorganisms. There are various techniques of minimising food losses after harvesting. Various storage methods have been advanced in order to extend the useful marketing distances and holding periods for fresh horticulture crops after harvest. Some of these methods include the maintenance at low temperature, modifying atmosphere packaging and controlling atmospheric storage. Maintaining the quality of fresh produce is the greatest challenge for food industries due to each method having its own advantages and disadvantages.

In recent years, using the technique of edible coatings to extend the shelf life of foods have gained a lot of attention. Edible films/coatings are the thin layers of edible materials which are applied evenly on the surface of food and it can be consumed with the food product. These coatings can be made by even single or by combining one or two components extracted from the agricultural waste thus replacing the waxy or synthetic coatings. Polysaccharide based coatings reduce the respiration rate of foods and provide great barriers to fats, oils, oxygen, and flavours at low humidity. In addition, these coatings provide a great barrier to gasses, water vapour permeability and microorganisms. Many polysaccharides are accessible in abundance from biodegradable agricultural sources like cellulose and starch. Preparing films from these sources or using their derivatives make the films having excellent packaging characteristics. Edible coatings differ from other conventional packaging materials due to their edible and biodegradable properties thus representing a unique category of packaging materials. Coatings preserve the fruits and vegetables by isolating the coated product from environment by generating modified atmosphere conditions. By modifying atmosphere conditions, the oxygen availability is reduced and the internal carbon dioxide concentration of fruits and vegetables is increased(Smith et al., 1987). Basically, coatings traps the carbon dioxide gas physically within the fruit tissues. This mechanism leads to higher levels of carbon dioxide which lowers down the respiration rates, and hence delay the senescence period by lowering the levels of ethylene production which acts as a ripening hormone in fruits and vegetables. In addition, coatings have different levels of oxygen permeability. This functionality of coating reduces the respiration and extends the shelf life. Films also decreases the production of aromatic volatile compounds by inducing anaerobic conditions and also reduces the off-flavours development which is due to anaerobic fermentation of sugars in fruits(Matthesis and Fellman, 2000). These also inhibit water loss from transpiration and are hydrophobic barriers. Hence, this feature is very useful for commodities like fruits and vegetables. To further extend the food stability, quality, safety and functionality, the coatings can be composed with additional functional materials like nutrients, flavours, antimicrobials and antioxidants(Krochta and De Mulder-Johnston, 1997).

After China, India is the second greatest producer of wheat producing country of the world. India is also the greatest producer of many agricultural byproducts besides wheat such as wheat straw, wheat husk, corn straw, rice straw, sweet potato, sugarcane etc. Largest quantity of agricultural residue is either used for animal feed or burnt after harvesting causing environmental pollution. this pollution creates the problems for living beings which is responsible for many of the diseases and harms the living beings. The large amount of wheat straw is produced while postharvestingwhich can be used as primary source of various bioactive products, valuable polysaccharide and value added products. Wheat straw mainly comprises of cellulose that is 30-40% and hemicellulose that is 20-35% and lignin that is 15-20%.

Arabinoxylan(AX) is hemicellulose present in both the primary and secondary cell walls of plants. It is also found in cereal grains and wood. It comprises of copolymers of two pentose sugars arabinose and xylose (McCartney et al., 2005). Arabinoxylan chain consists of huge number of 1,4-linked xylose units. Many of the xylose units are substituted with 2,3-linked arabinose residues(Dervilly et al.,2004). Arabinoxylan primarily provide the structural role in plant cells. They are also the source of extensive amounts of phenolic acid and other ferulic acids which are covalently linked to them. Phenolic acids functions as the defence and protecting elements against fungal bacterium. Arabinoxylan can also be used as an important source of dietary fiber and linked with different health benefits(Aguedo et al., 2014). Cereal grains are the staple foods consumed worldwide. Whole cereal grains are abundant source of dietary fibre. These dietary fibres inhibit disease incidence because they have large number of health benefits.

The cell wall of plants is made up of cellulose mainly. Cellulose is an organic component found in abundance in nature. In plants, it is responsible for primary building component and besides this it have many more uses. Cellulose is tasteless, does not have any odour. It is not miscible with water and other organic solvents. It is biodegradable in nature. Cellulose consists of hydroxyl groups which helps in producing cellulose derivatives such as cellulose esters or ethers through the reaction of various reagents.

OBJECTIVE

- Extraction of polysaccharides(i.e. Arabinoxylan (AX) and cellulose) from wheat straw and its carbohydrate estimation.
- Preparation of fatty acid esterified cellulose.
- Development and characterization of composite films (AX-cellulose fatty acid esters) derived from polysaccharides.

Review of Literature

Edible coatings are characterised as a thin layer of edible material which can be consumed with the food material and implements a barrier to oxygen, humidity, moisture and solute movement for the food. In recent years, edible films have gained certain advantages over synthetic films. The coatings are composed from renewable and edible materials and hence degrade more readily as compared to polymeric materials. Further, organoleptic properties of packed foods can be enhanced. These films acts as carriers for antimicrobial and antioxidant agents. These prevent the loss of nutrients from the food. basically, the aim of coating is to provide quality products having good nutritional, sensory and textural properties to the consumer. It could be primary packaging made from edible materials. In this process, thin layer of edible material can be directly applied on the surface of food and can be used as a food wrap without even changing the original product or processing method.

TYPES OF EDIBLE COATINGS

The three main ingredients used to make edible coatings or films are the edible polymers like polysaccharide, lipid, and the protein. In many examplification , two or all of these ingredients are blended to produce composite edible films(Hernandez-Izquierdo and Krochta 2008). Polysaccharide based edible coatings are generally hydrophylic and produces strong hydrogen bonding which is useful in cross linking with functional additives like colors, flavours, and micronutrients. Protein films provides good mechanical strength which minimises the injuries during transportation and in addition, these are also hydrophylic(Janjarasskul and Krochta 2010). Due to their hydrophylic property, these films provide poor moisture barrier. Lipid based coatings on the other side provides good moisture barrier and have hydrophobic structures due to which these films have low mechanical properties.

➢ POLYSACCHARIDE BASED FILMS

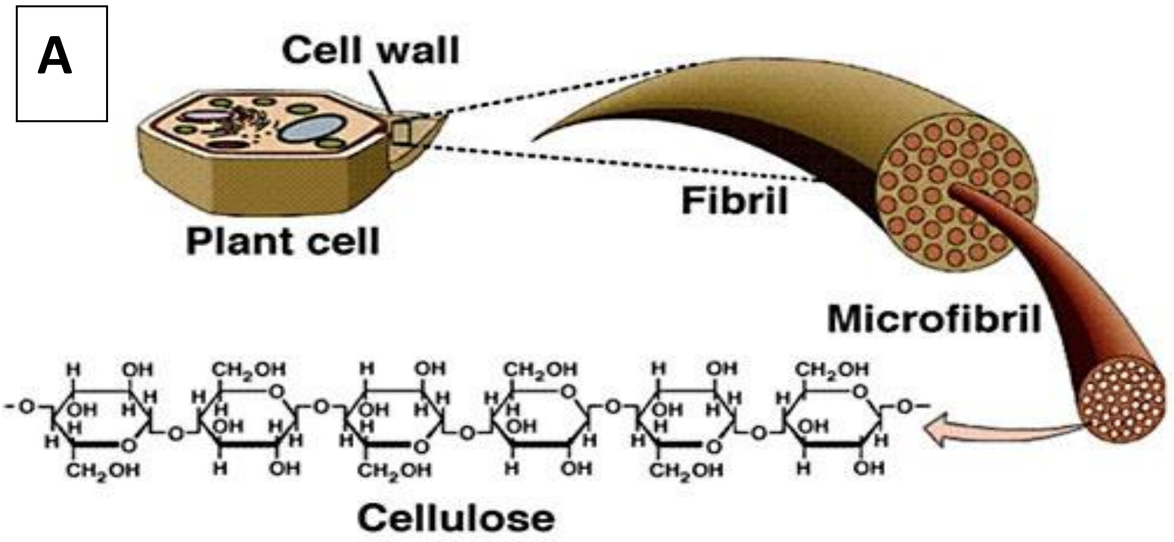
Polysaccharides consists of monosaccharide or disaccharide subunits. The main domain of polysaccharides used for edible films or coatings include cellulose, starch, pectin derivatives, chitosan, alginate, xylans and many more(Krochta and Mulder-Johnson,1997). These films are great barrier to oil and oxygen because the hydrogen bonds present in their subunits interact with the other hydrophyllic subunits to form a repetitive polymer. It is beneficial for food industries to make polysaccharide films due to availability of numerous sources, low cost, and relatively simple process. Following describe some examples of this type of ingredents.

Cellulose and derivatives

Cellulose is a natural polymer found in abundance and is widely utilized to produce biodegradable films. Because it is extracted from plants and present in abundance, it is of low cost, non toxic and a biodegradable component. It can also be synthesized from microorganisms. Cellulose havehighly crystalline structure due to which it is insoluble in aqueous state. Water solubility can be increased by treating cellulose with alkali to swell its structure, followed by the reaction with chloroacetic acid, methylchloride or propylene oxide to yield carboxymethylcellulose(CMC), hydroxypropyl cellulose(HPC) , methyl cellulose(MC). These films are tasteless and odourless having moderate tensile strength, barriers to oils and moisture, and have flexibility(Krochta and MulderJhonson, 1997). Esterified cellulose films have higher water vapour permeability. Both MC and HPC have the capability to produce thermally induced gelatinous coating. In addtion to this, they have been utilised to decrease oil absorption in deep frying foods (Kester and Fennema, 1986., Balasubramanium et al., 1997). MC could be utilised as coating material for confectionery products and can function in resisting lipid migration(Nelson and Fennema, 1991).

Starch and derivatives

Starch is present in tubers, legumes, cereals. It has many applications and is widely utilised in food industries due to its presence in abundance. Starch is utilised to develop biodegradable films thus replacing synthetic polymers. It provides great mechanical properties(Xu et al., 2005). Starch consists of two components that are amylose and amylopectin which are responsible for their solubility in water. Preferably , high amylose starch is used as a good source like corn starch for producing film. Starch alone is not enough to provide good mechanical properties but when mixed with plasticizers , by modifying it chemically or genetically or by combining both approaches. Starch based edible coatings arehydrophylic in nature. Starch based edible films have many advantages which includes its low cost, available abundantly, is a barrier of lipids and gases but as it is hydrophylic in nature, it has poor resistance against water. It can be utilised as a packaging material for candies and bakery food items(Bravin and others 2006). These films are brittle in nature and to overcome this drawback, certain plasticizers are mixed with starch like glycerol, urea, or polyhydroxycompounds having lower molecular weight. Plasticizers retard the microbial growth by reducing water activity. Some derivatives of starch such as cassava starch have been used for studying its barrier and functional properties by coating the film on fresh cut apples. It was observed that using cassava based coatings reduced the respiration rate. These films in addition exhibited higher antioxidant activity.



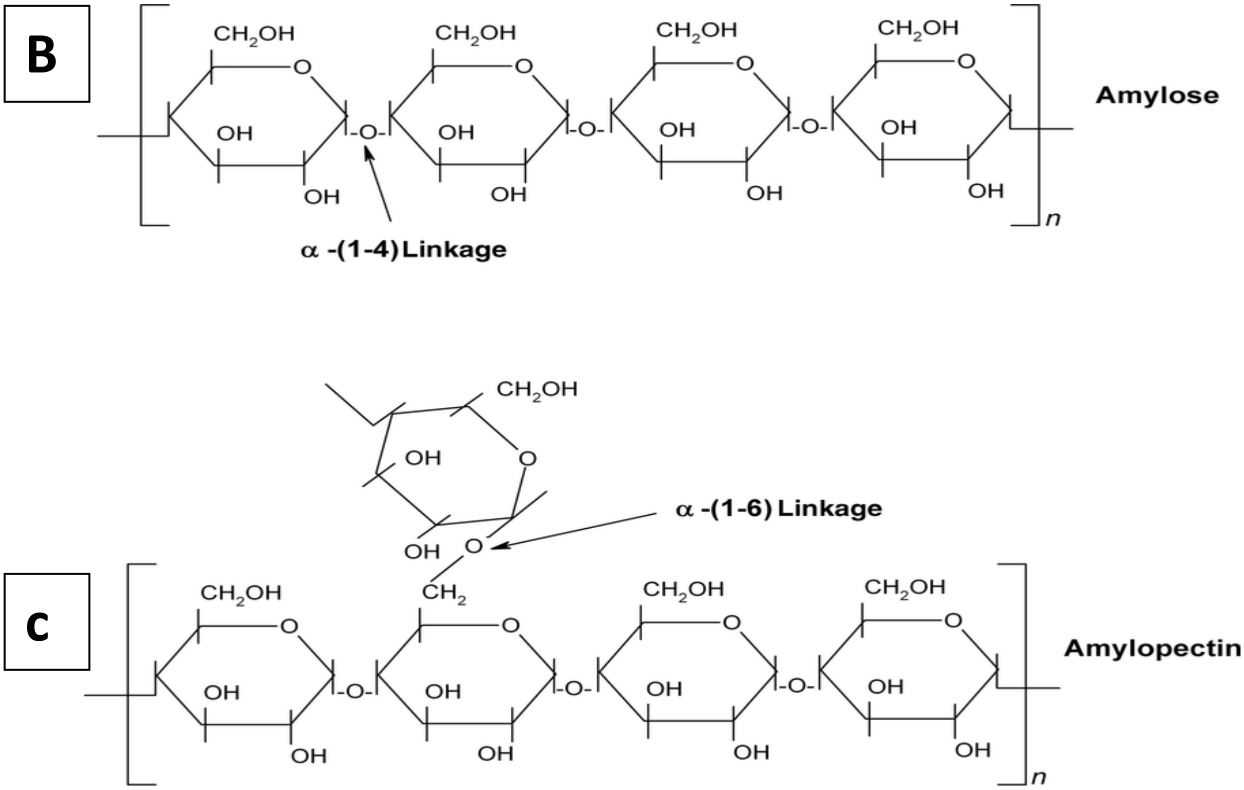
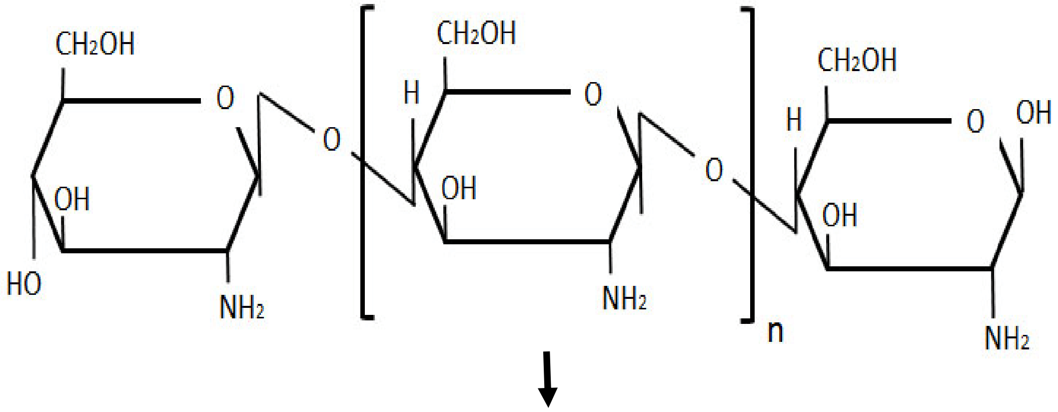


Figure1.1. The structures of polysaccharides (A) Arrangement of cellulose within the cell wall and (B) Chemical starch with amylose and amylopectin units.

Chitin and chitosan

After cellulose, chitin is the second most abundant biopolymer in nature. It is present in fungal cell walls, exoskeleton of crustaceans and other biological materials(Andrady and Xu,1997). It is mainly poly (β-(1-4)-2-acetamide D-glucose), which is structurally similar to cellulose except that secondary hydroxyl on the second carbon atom of hexose repeat unit is replaced by an acetamide group. Chitosan is derived from chitin and it is a linear polysaccharide. It is obtained by treating shells of crustaceans like crabs, crawfishes, shrimps with alkali treatment or with hot water treatment. Chitosan makes the film semipermeable due to which films atmosphere's get internally modified and results in delayed ripening and lowering of transpiration rates in fruits and vegetables. Films developed from aqueous chitosan are tough, clear in appearance, show flexibility and are barriers to oxygen(Sandford, 1989; Kaplan et al., 1993). When these polymers are methylated, the carbondioxide permeability gets improved. these coatings function as antimicrobial coating when coated on cucumbers, strawberries, bell peppers (El-Ghaouth et al., 1991a, 1991b) and provides barrier to gasses when coated on plums, apples, peach, pears (El-Ghaouth et al., 1991a, 1991b). In addition these, chitosan coatings are also used to extend the shelf life mango, capsicum, banana. It activates the enzyme called chitinase which acts as plant defence enzymes.



β-(1,4)-D-glucosamine

FIGURE.1.2. Structure of chitosan showing β-(1,4)-D-glucosamine linkage

Pectin

This polysaccharide is anionic and comprises of acid residues of β-1,4 linked d-galacturonic acid residues. High methoxypectinsleads to outstanding coatings or films. But when plasticizers like citrus pectin are added to high amylase starch, it develops more flexible and strong films. These films become thermal stable at 180^{0}C . In almost every proportion, pectin is soluble in poly alcohols. These films are commercially utilised as water miscible pouches for insecticides and detergents, also used as liners and bags which are flushable. Using plasticizer like glycerol or lactic acid, laminated films of both chitosan and pectin are formed (Fishman et al., 1994).

➢ PROTEIN FILMS

Proteins generally are of two types either fibrous or globular. Fibrous proteins are insoluble in water and plays important role in animal tissues serving as main structural material whereas globular protiens show their soluble nature in water or even sometimes in acids, salts, base solutions and serves in living systems(Scope,1994). Fibrous proteins are lengthy and are linked to each other parallely forming fibres through hydrogen bonding The globular proteins have complex spherical structures folded together through mixture of bonds like ionic, hydrogen bonds and many more(Scope, 1994). Many of the proteins like soy protein, whey protein, corn zein, have film producing properties. These films are hydrophylic generally and thus is affected by temperature and humidity. For developing such type of films, solutions or protein dispersions are used(Kester and Fennema, 1986). Generally, extended structures are needed to develop the film. To fulfil this requirement, the denaturation of protein must be done by action of heat, using solvent, acid or base. When the structures gets extended, the protein chains starts linking up through hydrogen, hydrophobic ,covalent and ionic bondings. This chain-to-chain linkages develops cohesive films which gets overwhelmed by amino acid residues sequence, their nature and degree of chain expantion. Higher chain-to-chain linkages leads to stronger films but with decreased gas permeability, liquid and vapours permeability along with reduced flexibility(Kester and Fennema, 1986). The following are the examples of films developed from different types of proteins

Gelatin films

Gelatin is extracted from protein called collagen by its controlled hydrolysis. Collagen is the fibrous protein which is insoluble in water. It is abundantly present in nature as the main element of bones, skin, and connective tissues. Basically, gelatin comprises of particular amino acids sequence. The aqueous solutions of gelatin remains in sol state at temperature 40^0 C approximately and when allowed to cool, physical and thermo reversible gels are formed. To produce gelatin film, gelatine in the concentration of 20-30%, plasticiser around 10-30% and water in the amount 40-70% are utilised and are further pursued by drying the gel(Guilbert, 1986). Gelatine is suitable for encapsulating the lower moisture or oil based food materials and pharmaceuticals. This encapsulation helps in the prevention of oxygen and light and along with this , it also helps in defining drug dosage (Gennadios et al., 1994). These coatings were also used to coat meats to prevent moisture, oxygen and oil migration (Gennadios et al., 1994).

Corn zein films

Zein is the main constituent of corn. This protein has higher amount of proline content thus it gets dissolved easily in ethanol(70-80%) (Dickey and parris, 2001,2002; Landry, 1997). It has hydrophobic characteristics and is also thermoplastic. Its hydrophobic property is due to higher content of amino acids which are non polar (Shukla and Cheryan, 2001). Zein has brilliant film developing properties and is applicable for fabrication of edible films. Plasticizers are added to increase the flexibility thus reducing the brittleness of film (Park, 1991). In contrast to other types of edible films, these films provide best water vapour permeability (Guilbert, 1986). Further addition of fatty acids or sometimes even addition of cross- linking reagents results in enhanced water vapour permeability. But addition of these components, edibility of films are affected (Alikonis, 1979). Park et al., observed that these coatings have capablility of reducing moisture and prevented loss in fruits firmness and also delayed colour change in tomatoes (Park et al., 1994b).

Wheat gluten films

Wheat gluten comes under the globular proteins. Wheat flour proteins which are not soluble in water describes the wheat gluten. It comprises of combination of polypeptide molecules. Gluten have certain characteristic like those of cohesiveness and elasticity which aids in film development. It consists of two fractions of proteins of wheat flour which are prolamine and glutelin which are further introduced as gliadin and glutenin. Unlike glutenin, Gliadin gets dissolved in 70% ethanol (Gennadios and Weller, 1990). Though wheat gluten is water insoluble, it readily dissolves in aqueous solutions attaining higher or lower pH (Krull and Inglett, 1971). By drying wheat gluten aqueous solution in ethanol leads to formation of film (Gennadios and Weller, 1990).To enhance the flexibility of film, glycerine like plasticizer is added (Gennadios et al., 1994). But when sorbitol content was increased to enhance the flexibility, it was observed that sorbitol lead to decreased elasticity, strengthand also reduced the water vapour permeability (Gontard et al., 1992). However, (Herald et al., 1995) reviewed that the films were stronger and clearer when gluten of greater purity was utilised. A review on size of plasticizer was done by (Herald et al., 1995) and he observed that using spray-dried gluten, films developed were more stable as compared to films developed by using flash dried wheat gluten. When coated on egg shells of grade A quality, the quality was extended for 30 days. Various methods were observed to enhance the tensile strength of films like utilising cross linking reagents like glutaraldehyde or by exposing heat at 80^0 C (Gennadios and Weller, 1992; Koelsch and Labuza, 1992).

Soy protein films

Cereal grains have protein content around 8-15% but protein content of soybeans is much higher than that i.e. about 38-44%. Soybeans protein gets dissolved in diluted neutral salt solutions but it is not soluble in water. They come under the category of globular proteins (Kinsella, 1979). According to sedimentation rates, its protein is differentiated into 2S, 7S, 11S, 15S fraction (Gennadios et al., 1994). The fundamental elements are 7S and 11S fractions and they have quaternary structure (Kinsella et al., 1985). Soy protein is rich in residues of asparagine and glutamine. It has two tightly folded proteins i.e. conglycinin and glycinin. There are two methods for forming film either by forming surface film by heating soymilk or by forming film from isolates of solutions of soy protein (Gennadios and Weller, 1992). Soymilk is formed by milling soybeans along with water and then the milk is separated from soybeans.

Casein

Casein is the protein derived from milk. It has coil structure due to which it becomes easily accessible. But when processed with particular plasticizers at $80\text{-}100^0$ C temperature, the mechanical properties are enhanced and thus films vary from brittle to tough and stiff ones become flexible. This protein makes the film convenient for film blowing because it is hugely stretchable. Such type of films are opaque in nature. These are insoluble in water, but when immersed for 24 hours, casein materials gained weight upto 50% approximately. The main disadvantage of using casein is that it is expensive.

Keratin

Keratin is obtained from nails, hairs and feathers like waste products. It is very cost friendly material. As it consists of huge groups of cysteine, it is not easily processible. But when processed, water insoluble plastic is formed which is biodegradable in nature. This protein based films posses the poor properties in contrast with other types of proteins. Keratin can not sense the humidity and to overcome this limitation, it is either laminated or blended.

➢ LIPID FILMS

The films developed from lipid based materials inhibit moisture migration from fruits and vegetables. These films show great resistance to moisture because of their low polarity. Several lipid compounds are used for developing films such as natural wax and surfactants. . But lipids are hydrophobic in nature so they develop the thicker and brittle films in contrast. To overcome this drawback, biopolymeric materials are added or combined with lipid compounds to produce film. To enhance the functional and mechanical properties of these films, protein like agents are mixed(Debeaufort et al., 1993).

Fatty Acids and Monoglycerides

Fatty acids are obtained from the vegetable oils. When glycerol and triglycerol are trans-esterified, monoglycerides are formed. These types of films are most importantly utilised as emulsifiers and sometimes as dispersing reagents (Hernandez, 1991).The acetylated monoglyceride can solidify from molten state to wax like solid which has flexible property. At solid state, various lipids are stretchable before fracturing upto 102% to their original length but if compared with acetylated monoglyceride, then it is stretchable upto 800% to its original length (Jackson and Lutton, 1952). Such type of films have lower water permeability than polysaccharide based edible films excluding methyl cellulose.

Waxes and paraffin

Paraffin wax is derived from distillate fraction of crude petroleum and it comprises of combination of solid hydrocarbon. It is permitted to coat on raw fruits, vegetables. Wax based films provide barriers to gas and moisture. These type of films makes the fruits and vegetables to look appealing. It prevents the migration of moisture from fruits(Bourtoom, 2008., Dehghani et al.,2018.,Hassan et al., 2018). Wax based films are considered edible only if it is coated in thin layer otherwise, it is removed before consumption. Carnauba wax is also used for developing wax based coatings or films.

Shellac resins

These are resins comprises of complex which is the combination of polymers of aliphatic alicyclic hydroxyl acid. Basically shellac resins are the secretions secreted by Lacciferlacca insect. These resins show the solubility in alcohols and sometimes in alkaline solutions. These types of coatings are widely utilised in pharmaceutical industries to coat the product (Hernandes, 1994). But Hagenmaier and Baker observed that resins and their derivatives are in wide use to coat citrus like fruits. The purpose of designing such type of coatings or films was to impart its high gloss property to attract the buyer during inspection. Different effects are shown by this film towards gas transmition or exchange like properties(Hagenmaier and Baker, 1993). In contrast to wax coatings, these coatings when coated on citrus fruits showed decreased internal oxygen, increased internal carbon dioxide and it elevated the ethanol content in fruits. Whereas, elevated ethanol content leads to indication of flavour(Ahmad and Khan , 1987., Cohen et al., 1990., Ke and Kader, 1990).

FIGURE 1.3. Image of carnauba wax

FIGURE 1.4. Image of bees wax

FIGURE 1.5. Image of shellac wax

Materials and Methods

1) EXTRACTION OF ARABINOXYLAN(AX) AND CELLULOSE FROM WHEAT STRAW

Equipment used

1. Beakers
2. Rotary-vapor
3. Fume hood
4. Lyophilizer
5. Centrifuge
6. Dialysis tube
7. pH meter
8. Mass collider

Chemical used

NaOH solution (0.2M), $NaBH_4$, Acetic acid. All chemicals were bought from Sigma Aldrich.

Detailed Procedure

1. 500g of wheat straw was washed with MQ water and dried in oven at 60^{o}C.
2. Then it was milled in mass collider to make it in powdered form.
3. After this, the powder was treated with NaOH (0.2M) solution containing sodium borohydride (gm/l) in ratio of 1:15 and was heated at 80^{o}C for 2 hours.
4. Then it was centrifuged at 8000 rpm for 10 mins.
5. The pellet and supernatent were seperated.
6. The washing of pellet was done with water and kept for drying in oven till the moisture was removed completely. The dried residue contain mainly cellulose.

7. Using glacial acetic acid, the supernatent was neutralized and was kept overnight.
8. Then it was concentrated in rotary vapour.
9. The dialysis tubes were taken and slurry was poured in it and was kept in bucket filled with RO water for 4 days.
10. This water was changed after every 6-7 hours.
11. Further, it was dried in lyophilizer jars by freeze-drying in lyophilizer.
12. In air tight container, the Arabinoxylan powder was preserved.

2) CARBOHYDRATE ESTIMATION BY PHENOL SULPHURIC METHOD

Equipment used

1. Test tubes
2. UV spectrophotometer
3. Well plate

Chemical and other material required

Sample containing AX, 5% phenol, Sulphuric acid, Standardized sample of xylose, glucose and cellulose, ethanol, crushed ice, milli-Q water.

Detailed procedure

1. In a glass bottle, 5% phenol was prepared.
2. The solutions of xylose and wheat straw AX were made in the concentration of mg/ml.
3. In test tubes, the samples of AX and xylose were taken in the concentration of 50µl, 100µl, 200µl, 300µl, 400µl, 500µl.
4. Then, MQ water was added in the concentration of 450µl, 400µl, 300µl, 200µl, 100µl.
5. In every tube, 250µl of 5% phenol was added respectively and further 2ml of sulphuric acid was added.
6. The test tubes were kept in crushed ice for 10 mins for incubation and were vortexed
7. At 490nm, the absorbance readings were taken.
8. the amount of total carbohydrate content present in solutions were calculated from standard caliberation curve(calculation vs OD plot).

Calculation

Absorbance relates to 0.1ml of the test=

x mg of glucose in 100ml of the sample contain = (x / 0.1) X 100 mg of glucose

=% of total carbohydrate.

3) COMPOSITIONAL ANALYSIS BY GC-MASS SPECTROPHOTOMETRICALLY

Chemicals used-

1. Sample of AX
2. 2M Trifluoro acetic acid (TFA)
3. Inositol
4. Ammonia
5. Isopropyl alcohol (IPA)
6. Acetic acid
7. Methanol
8. Sodium borohydride
9. Pyridine
10. Toluene
11. Dichloromethane
12. Glacial acetic acid
13. Phosphorus pentoxide

Methodology

1. In the concentration of 1-5mg sample, 100µl of inositol was added along with 2-3µl of TFA and was heated for 2 hours at the temperature 120^0C.
2. Then, it was allowed to cool at room temperature.
3. TFA was allowed to evaporate in rotary evaporator in pear shaped flak.
4. 100% of isopropyl alcohol was added around 1ml and was re-evaporated.
5. 3 times washing was done with IPA to remove TFA fully.
6. 0.1mg/ml ammonia solution was added in concentration of 1ml till the pH of solution was nuteralised.

7. Then, 10 mg of $NaBH_4$ was added and was allowed to incubate for 16 hours at the room temperature.
8. Glacial acetic acid was added dropwise to neutralise the reduced solution and by using pH strips, the pH of solution was checked.
9. 90% methanol (10ml acetic acid : 90ml methanol) in concentration of 1 ml was used for washing 3 times in rotary evaporator.
10. Again, 100% of methanol in concentration of 1ml was added in pear shaped flasks and was rotary evaporised to dry the sample.
11. The samples were kept in dessicator over p_2O_5 for the duration of 2-3 hours.
12. In 50:50 ratio, 1ml pyridine was added along with 1ml of acetic anhydride for acetylation.
13. The samples were mixed by vortexing and the sonication was done for 30-40 seconds.
14. Mixture was heated at 100^0C for 1 hour and was shaken occasionally.
15. The mixture was allowed to cool at room temperature.
16. By using rotary evaporator, pyridine and acetic anhydride were evaporated.
17. Then, 1ml of toluene was added after complete drying and was vortexed and evaporated
18. 1ml of dichloromethane and water was used for dissolving the dried sample and was vortexed.
19. The crust got settled down at the bottom andin screw capped glass bottle tube, this layer was collected and using N_2 flush, dichloromethane was removed.
20. The sample was injected in GC column.

4) ESTERIFICATION OF CELLULOSE

(a) PREPERATION OF LAURIC ACID CELLULOSE ESTERS:

Equipment used

Magnetic heat stirrer, measuring cylinder, magnetic bead,1% NaOH solution.

1. 1gm of cellulose was taken in round bottom flasks.
2. 1% of NaOH (10 ml) was added to the flasks.
3. Then, it was mixed on magnetic heat stirrer for few minutes.

4. In flask 5ml of lauroyl chloride was added and the flask was heated on silicone oil for 4 hours at 110^{o}C.
5. After this step, ice water was used for precipitation of reaction mixture.
6. The precipitates were filtered using filter paper.
7. Washing was done with water, ethanol and acetone and were kept for drying at room temperature.
8. These dried precipitates were stored in sample container.

(b) PREPERATION OF STEARIC ACID CELLULOSE ESTERS

Equipment used

Magnetic heat stirrer, measuring cylinder, magnetic bead,1% NaOH solution

1. 1gm of cellulose was taken in round bottom flasks.
2. 1% of NaOH (10 ml) was added to the flasks.
3. Then, it was mixed on magnetic heat stirrer for few minutes.
4. In flask 5ml of stearoyl chloride was added and the flask was heated on silicone oil for 4 hours at 110^{o}C.
5. After this step, ice water was used for precipitation of reaction mixture.
6. The precipitates were filtered using filter paper.
7. Washing was done with water, ethanol and acetone and were kept for drying at room temperature.
8. These dried precipitates were stored in sample container.

5) FOURIER TRANSFORM INFRA-RED (FTIR) SPECTRA ANALYSIS

FTIR stands for Fourier transform infrared. It is the most adopted method for intfrared spectroscopy. It have interoferometer which helps in recording the information about sample placed against the IR beam. The patterns represented by spectra helped in identifying the sample

1. Infrared radiations were allowed to pass through the samples. Some of them was passed out of sample and some rays were absorbed by the sample.
2. The resulted signal represented the fingerprint of the sample by the detector.
3. Different samples having different chemical structure represented the different fingerprints.

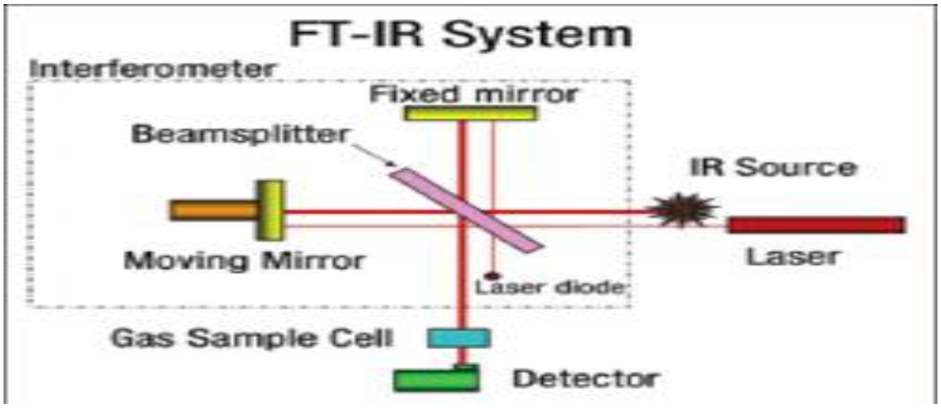


FIGURE1.6. Operation of FTIR instrument

6) PREPERATION OF HOMOGENOUS FILMS

1. AX, AX-Cellulose(60:40, w/w), AX-LAC(60:40,w/w), and AX-SAC(60:40,w/w) were solubulised in deionised water: EtOH (80:20) to form homogeneous films.
2. These solutions were then homogenised with probe sonication homogeniser.
3. After homogenising, glycerol was diluted in concentration of 20% of lipid phase and in addition, 3% of Tween-80 was added as emulsifier.
4. Then, these solutions were casted in film assemblies made with petri plates.
5. The assemblies were allowed to dry by keeping them in chamber having 23 ± 1^0C temperature and 50% of relative humidity for 3-4 days.

7) SCANNING ELECTRON MICROSCOPY (SEM)

SEM was used to characterize surface morphology of the films qualitatively.

1. Small pieces of films were cut.

2. The cut pieces were placed on holder.
3. Then, these pieces were coated with gold.
4. With the help of 10KV accelerating voltage and magnification at 500 and 1500X, the microstructures of coatings or films was observed.

8) WATER VAPOR PERMEABILITY RATE(WVPR)

Water vapor permeability of the films were examined by using gavimetric method.

1. Anhydrous calcium chloride was filled in the cups leaving 5mm gap from the top.
2. Vacuum silicone grease was applied on the edges of cups.
3. The circular films were placed on the top of the cups and were sealed with grease.
4. Then, the initial weight of these assemblies were noted down and were kept in conditioned chamber having temperature of 22 ± 1^{0}C and relative humidity of that chamber was 50 ± 1^{0}C.
5. The weight of the cups was noted down at the interval of 4 hours for 120 hours.
6. The change in weight was noted down and the graph was plotted for observation.

Calculations

WVTR = S/A

where S and A represents to slope of weight gain against time curve (g/day) and exposed film area (m^2).

Optical properties

To observe the reflecting spectra of films, a spectro-colorimeter was utilised. Using both white and black background, the absorbance of films was measured from 500-700nm.

RESULTS AND DISCUSSION

1) Extraction

The yield of Arabinoxylan was found to be 17% and of cellulose was 54%.

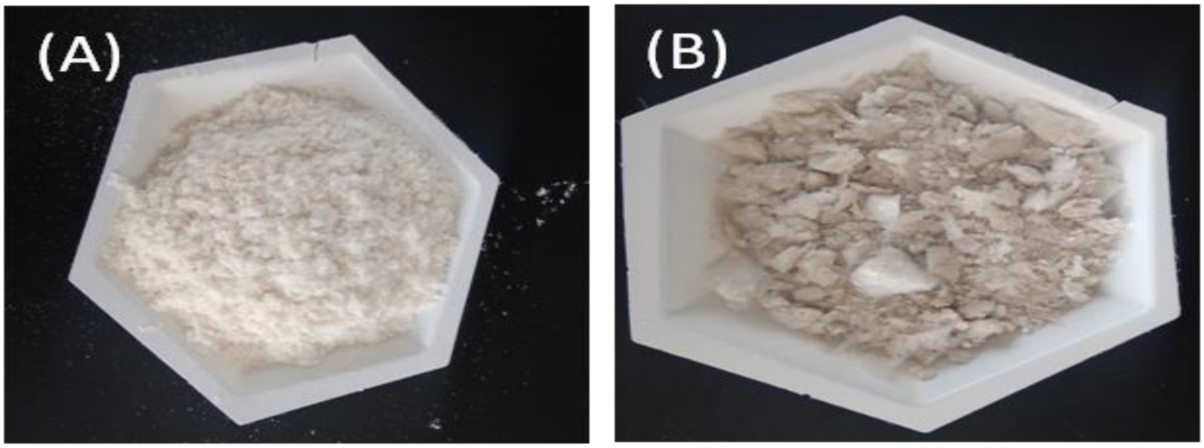


Figure1.7.(A)Wheat straw cellulose and (B)Wheat straw Arabinoxylan.

Sample type	Arabinoxylan	Cellulose
Yield (%)	17	54

2) Carbohydrate estimation by phenol sulphuric method

Using xylose, the curve was standardised and total carbohydrate content of Arabinoxylan was determined. The optical density of the sample was taken at 490nm absorbance.

Samples	Carbohydrate content (%)
AX	97 %
Cellulose	88%

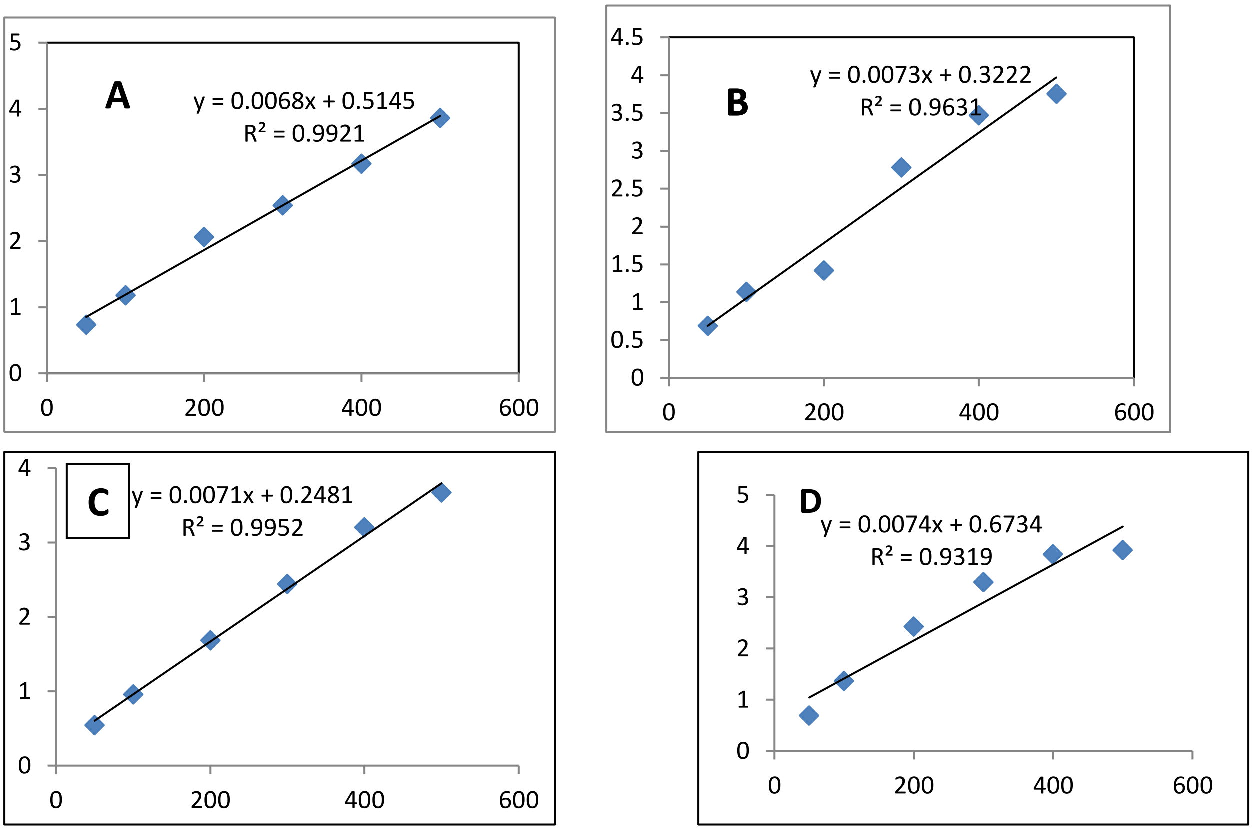


Figure 1.8. (A) Xylose standard graph, (B) Arabinoxylan graph for carbohydrate estimation (C) Standard graph of Glucose, (D) Cellulose for carbohydrate estimation.

3) Compositional analysis of extracted AX and Cellulose

GC-MS analysis of AX showed presence of arabinose and xylase as major constituent sugars whereas glucose was identified as predominant monosaccharide in cellulose

Sugar residue (mol %)			
Extracts	**Arabinose**	**Xylose**	**Glucose**
WAX	**13.31**	**86.68**	**-**
Cellulose	**-**	**-**	**100**

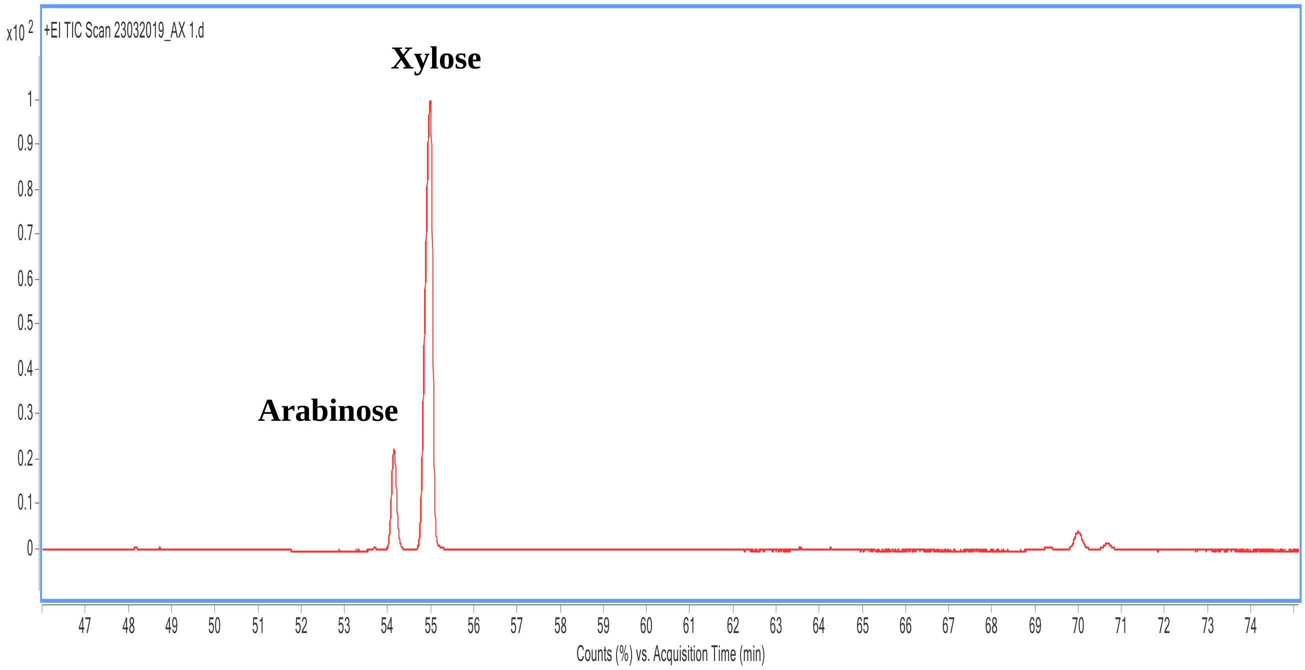


FIGURE1.9. GC-MS chromatogram of Arabinoxylan extracted from wheat straw.

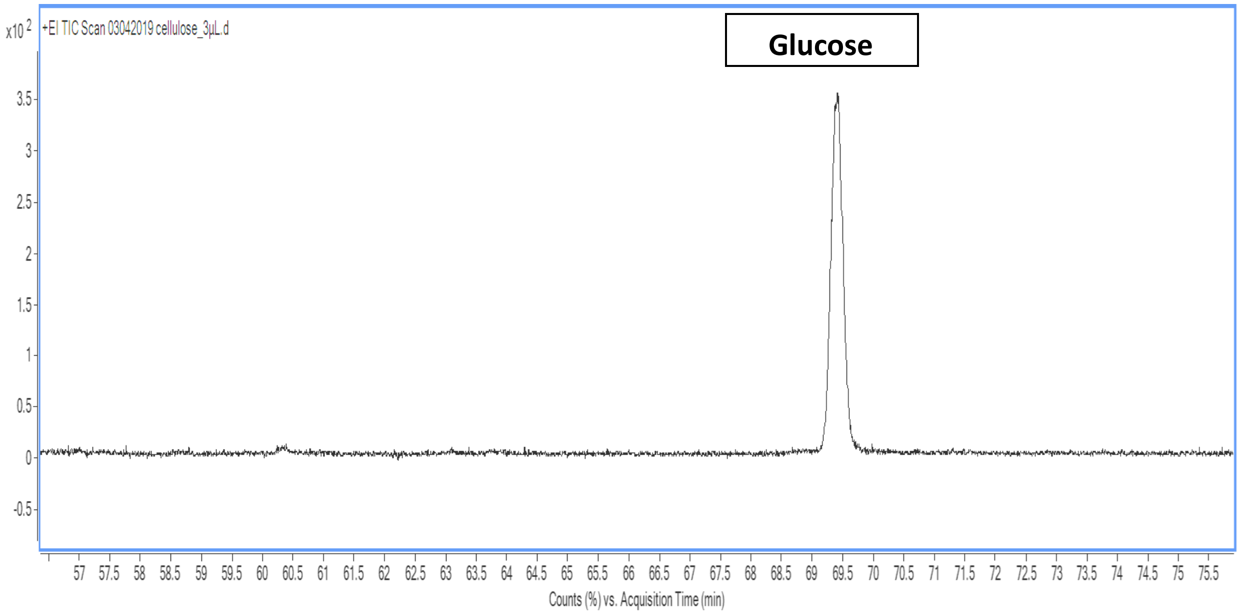


FIGURE2.0 GC-MS chromatogram of cellulose extracted from wheat straw.

4) Esterification

- Lauric acid esterified cellulose-

4.2 gm lauric acid esterified cellulose was prepared from 1gm cellulose and 5ml lauric acid.

Yield increase = 320%

- Stearic acid esterified cellulose-

3.7gm stearic acid esterified cellulose was prepared from 1gm cellulose and 5ml stearic acid

Yield increase = 270%

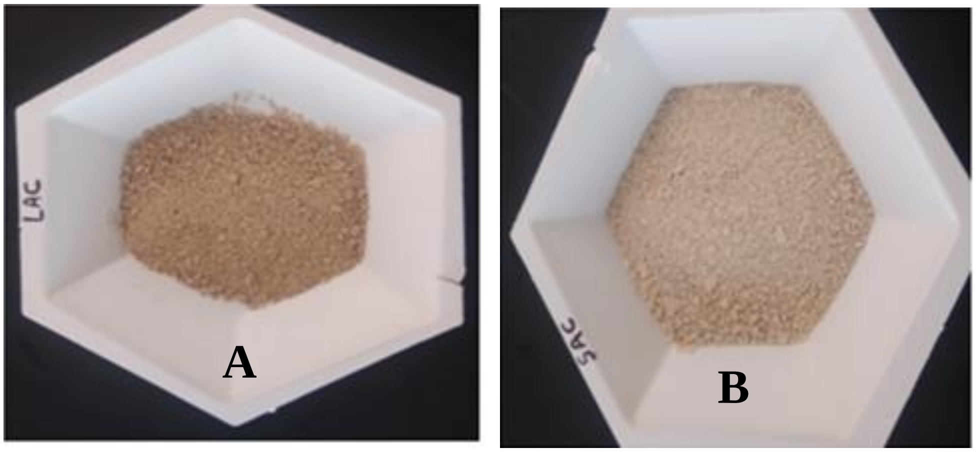
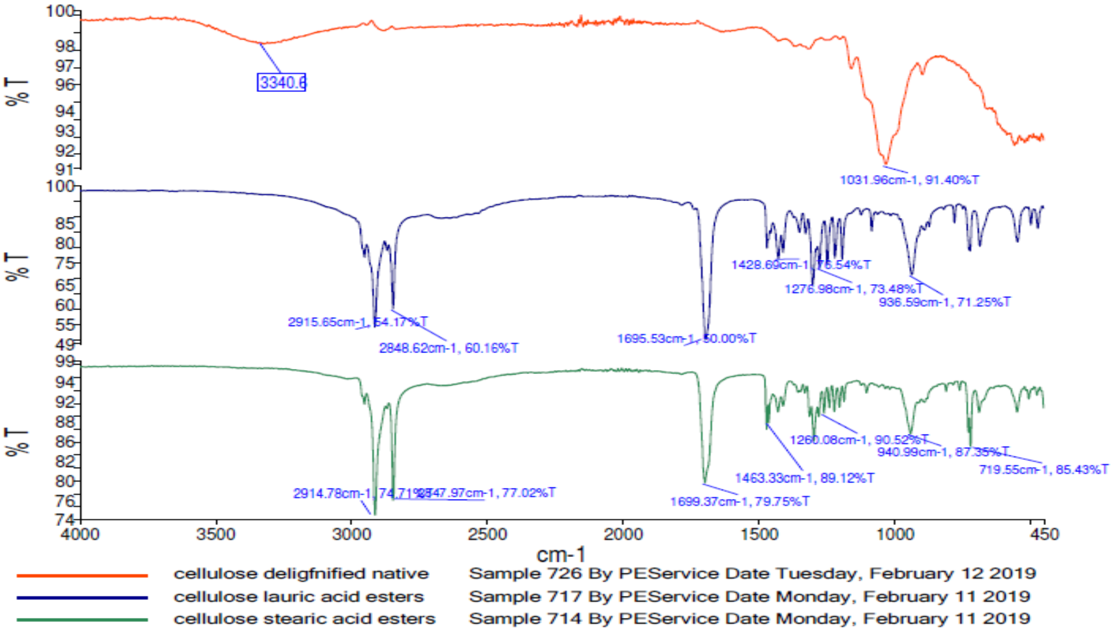


Figure2.1. (A) Lauric acid-cellulose ester (LAC) B) Stearic acid-cellulose ester (SAC).

5) Fourier Transform Infra-Red (FTIR) Spectra Analysis

Cellulose was esterified by fatty acid chloride (Lauroyl Chloride and Stearoyl Chloride) and NaOH mixture. Further, FTIR spectra were collected for the spectral characterization of fatty acid esterified cellulose. The presence of polysaccharide in the sample was confirmed by occurance of absorption band in the range of 1000-1200cm^{-1}. A sharp stretch peak in the region of 1699 cm^{-1} suggested the presence of C=O group of carboxyl ester moeity of esterified fatty acids. The bands in the region of 2848-2915 cm^{-1} was due to C-H variations mainly of fatty acids and glucose residues.

Figure2.2. FTIR spectrum of the native cellulose, cellulose-fatty acid esters (LAC: Lauric acid conjugated cellulose, SAC: stearic acid conjugated cellulose)



6) Preparation of Films:

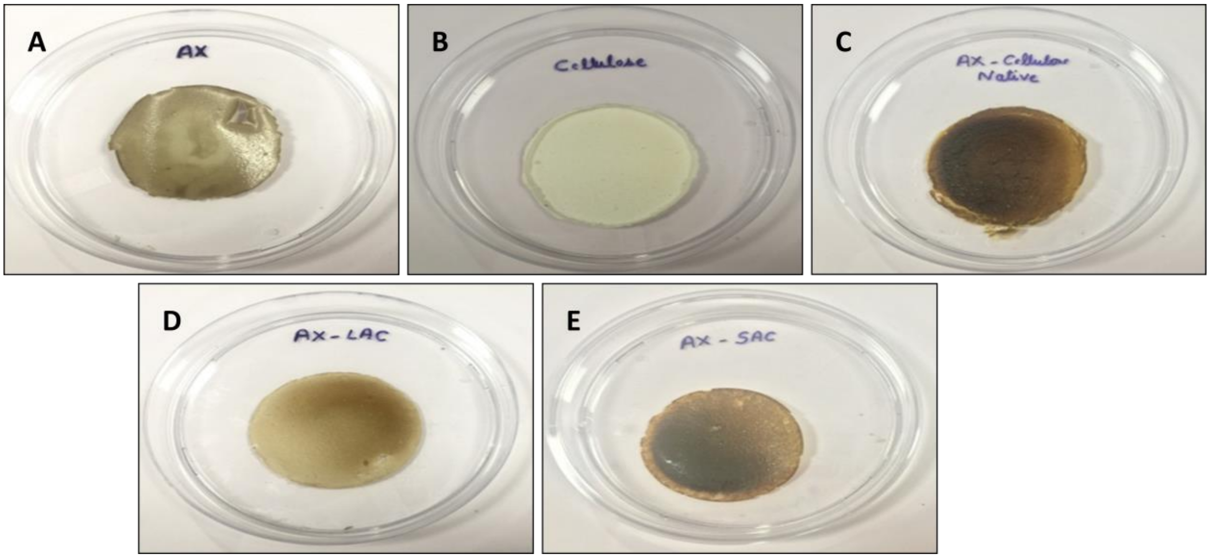


FIGURE2.3. (A) Arabinoxylan Film, (B) Cellulose Film, (C) Arabinoxylan-Cellulose Native Film, (D) AX-LAC and (E) AX-SAC.

7) Microstructural properties (SEM)

The film microstructural properties were determined by SEM to know surface morphology of dried film components. The AX-Cellulose film showed a smooth homogenous aspect with continuous surface in the matrix. Film with fatty acid conjugated cellulose (AX-LAC and AX-SAC) had laminar structures which were associated with growth of molecular aggregation of fatty acids during drying and crystallization of fatty acids in matrix. The length of the lipid layer were greater for AX-SAC and decreased as the chain length decreased, smaller lipid layers were observed for AX-LAC films. This suggested long chain fatty acid (SAC) having higher hydrophobicity was responsible for more effective growth of bi-layer self association of molecule aggregates.

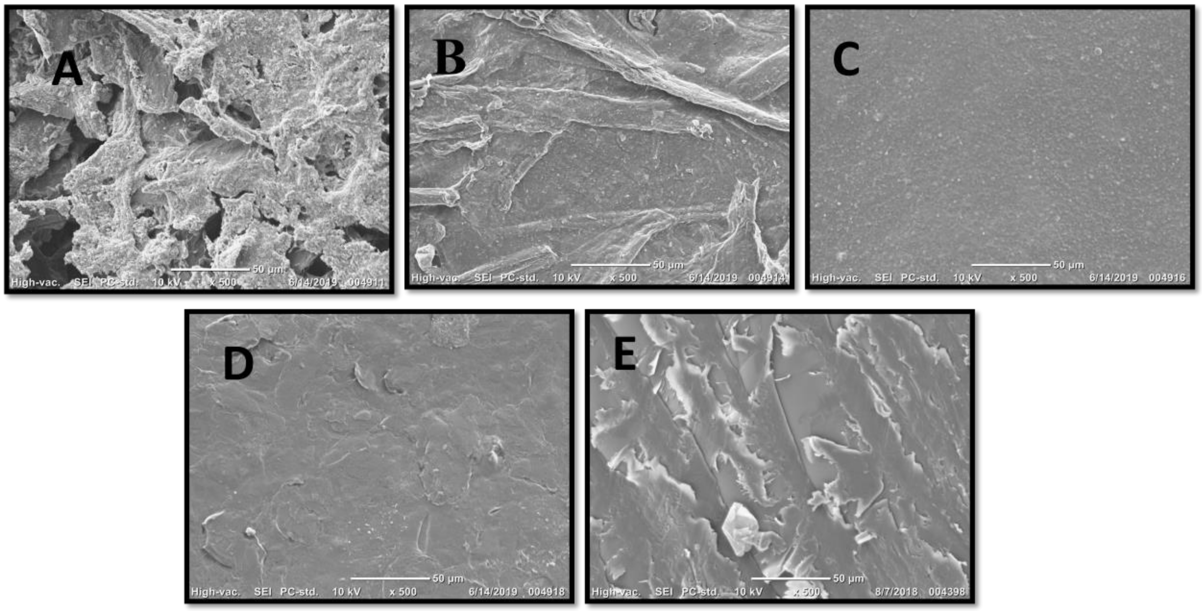


FIGURE 2.4. Microstructural properties of (A) Arabinoxylan film, (B) Cellulose film, (C) Arabinoxylan-Cellulose Native film, (D) AX-LAC film and (E) AX-SAC film.

8) Water vapor permeability

Water vapor permeability is related to amount of moisture migration from the film. Lesser the water vapor permeability value, higher moisture retention capability of films. Water vapor permeability of AX and cellulose were 6.8 g/m^2/day and 5.4 g/m^2/day suggesting the higher water vapor permeability of AX films and poor moisture barrier property. Further, addition of cellulose fatty acid esters to AX improved the moisture barrier property of the composite films (AX-Cellulose 60:40, 2%) as the WVTR value reduced to 5.7 g/m^2/day. AX-LAC (60:40, 2%) and AX-SAC (60:40, 2%) had lower water vapor permeability value of 2.8 and 2.3 g/m^2/day respectively suggesting improvement in the moisture barrier properties by 49% and 59% respectively compared to native AX-Cellulose (60:40, 2%) films.

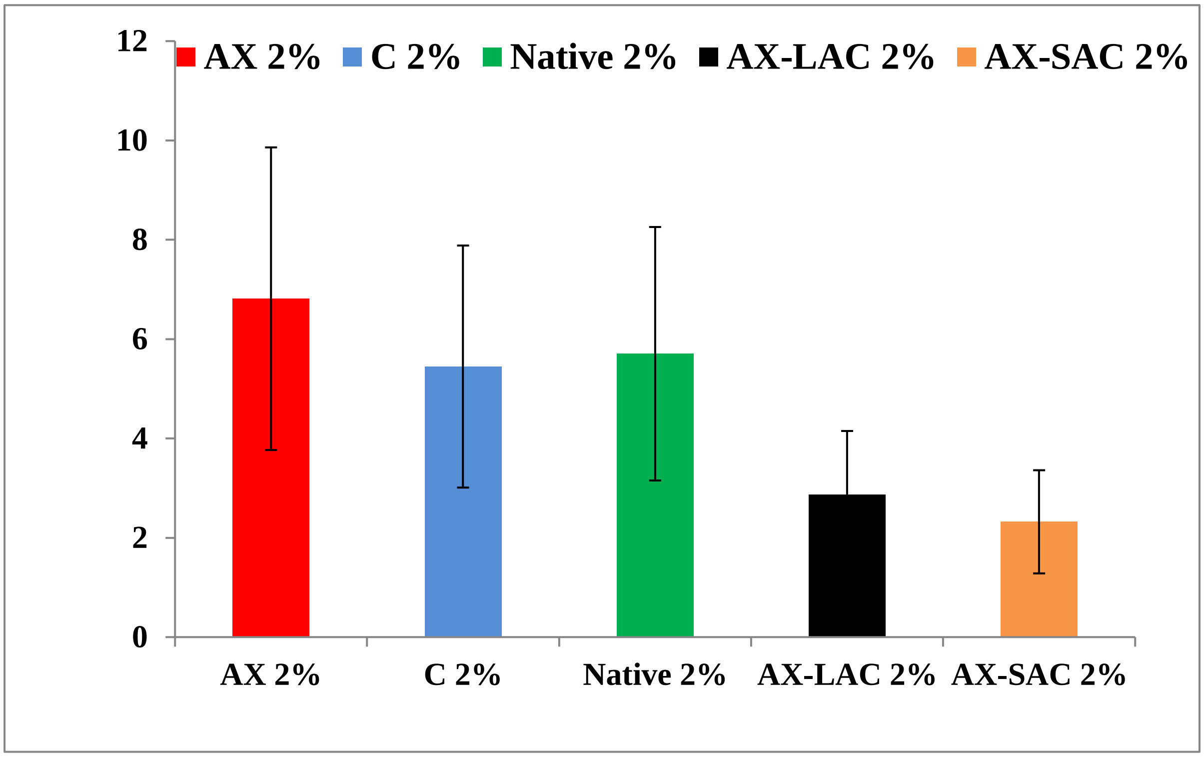
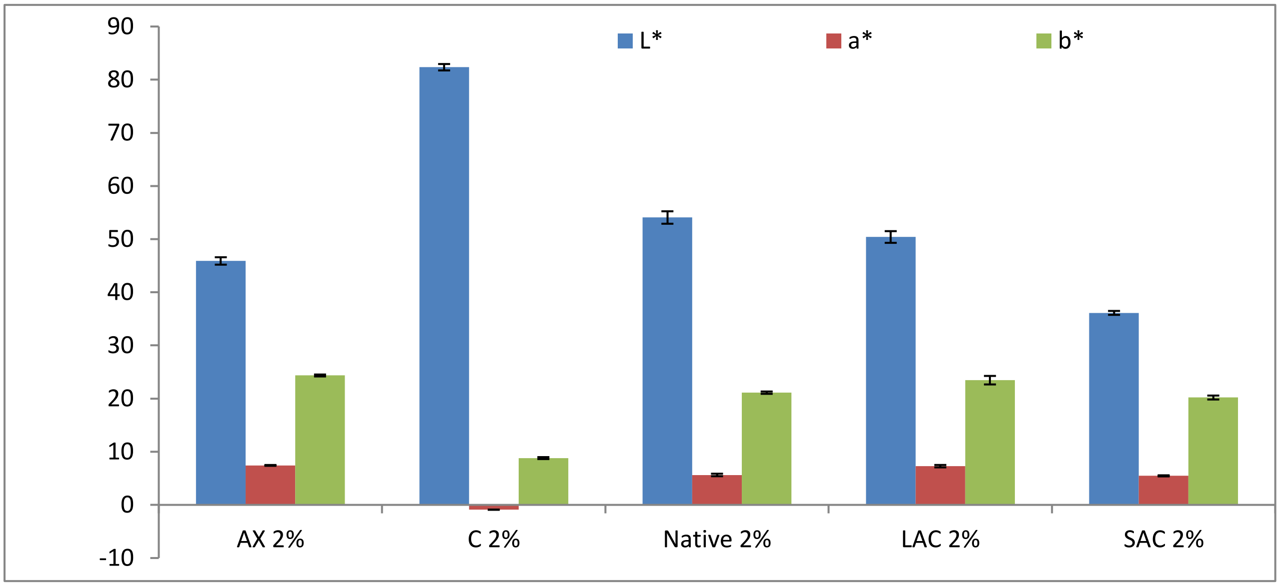


FIGURE 2.5. Graphical representation of water vapor permeability of film.

9) Color of films

Cellulose (2%) film had higher L* value whereas addition of AX to cellulose film caused reduction in the L* value. AX-Cellulose (60:40) composite films had L* value of 54 which might be due to phenolic compounds present in AX. Similarly, AX-LAC and AX-SAC (60:40, w/w) films exhibited L* values in the range of 36.12-50.14 which were comparable with the native AX-Cellulose film.



L* represents lightness

a* represents redness to green

b* represents yellow to blue

Conclusion

In this study, abundant biopolymers AX and cellulose were produced from agricultural by-products such as AX and cellulose were extracted from wheat straw. Further, cellulose was esterified with fatty acids to prepare fatty acid esterified cellulose (Lauroyl and stearoyl chloride esterified cellulose). The structural characterization of arabinoxylan (AX), cellulose and fatty acid esterified cellulose were performed using phenol sulfuric method, GC-MS and FTIR. Further, composite films were prepared by incorporating LAC and SAC (LAC: 50%, SAC: 59%) to AX based films. The microstructural and WVTR analysis suggested that the laminar structures in the AX-LAC and AX-SAC films had significant improvement in the moisture barrier properties causing nearly 50-59% reduction in water vapour permeability compared to native AX-Cellulose film. This study suggested potential application of AX-LAC and AX-SAC films. Coating material for preventing moisture loss from perishable fruit crops. However further detail studies on post harvest quality improvement of perishable fruits are required to substantiate the claims.

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