PECTIN FROM *LAGENARIA SICERARIA* AND ITS CHARACTERIZATION

Dissertation

Submitted in partial fulfillment of the requirement for

The award of the degree of

MASTERS OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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CERTIFICATE

This is to certify that the dissertation report entitled **"Pectin from *Lagenaria siceraria* and its characterization"** submitted by **Neha Sharma** (Roll. No 601604006) in the partial fulfillment of the requirement for the award of the degree of Masters of Technology in Biotechnology, Department of Biotechnology, TIET, Patiala, is a record of the student's own work carried out by her under my supervision and guidance. This dissertation fulfills the requirements as per the regulations of the university and meets all the necessary standards for the submission. This report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

Jyoti 12/10/18

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CANDIDATE'S DECLARATION

I, **Neha Sharma (601604006)**, a bonafide student of Masters of Technology in Biotechnology in TIET, Patiala, would like to declare that the declaration report entitled **"Pectin from *Lagenaria siceraria* and its characterization"** submitted by me in the partial fulfillment of the requirement for the award of the degree of Masters of Technology in Biotechnology, is my authentic record of my work which I have done in the period of one year under the supervision of Dr. Jyoti Rani, Assistant Professor (Food Technology), Department of Biotechnology, TIET, Patiala. This report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

Place: TIET, Patiala

Date: 12/10/2018

Neha Sharma

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Place: Patiala
Date: 12/10/2018

Neha Sharma

ABSTRACT

The method for pectin extraction from *Lagenaria siceraria* was optimized using acid extraction method as used by P. Srivastava *et al*., 2011. To optimize the pH 0.1 M of H_2SO_4 was used to maintain 1.5, 2.0 and 2.5 values with variation in time of incubation for 1 hr, 2 hrs and 3 hrs simultaneously the temperature was also optimized at 50^oC, 60^oC and 70^oC respectively. A significant effect was observed on the yield of the pectin percentage from *Lagenaria siceraria* to the control sample (apple pectin) with a gradual increase in 3.81% (pH- 1.5), 3.86% (pH- 2.0) and 3.86% (pH-2.5). The ascending pH variation was observed with a positive significant effect on equivalent weight (*Lagenaria siceraria* pectin-9.7, Apple pectin-2.3 & Standard pectin-0.5), methoxyl content (*Lagenaria siceraria* pectin-90.8, Apple pectin-1.4 & Standard pectin-1.6), total anhydrouronic acid content (*Lagenaria siceraria* pectin-1222.5, Apple pectin-1525.3 & Standard pectin-1391.6) and degree of esterification (*Lagenaria siceraria* pectin-17.9, Apple pectin-5.6 & Standard pectin-8.9). The pectin obtained from *Lagenaria siceraria* was found to be less in percentage to whole piece but in comparison to the quality for gel making quite promising nearby to that of apple pectin as observed through the FTIR and XRD results. It would not be wrong to say that the *Lagenaria siceraria* can be a cheaper source for pectin extraction but the quantity is less however overall quality can be exploited to provide best results.

Keywords: Pectin, *Lagenaria siceraria*, Acid extraction method, Equivalent weight, Methoxy content, Anhydrouronic acid content, Apple pectin, Degree of esterification, FTIR, XRD.

CONTENTS

CHAPTER-I

INTRODUCTION

Pectin categorized as hetero-polysaccharide as for extensive presence in the cell wall of fruits and vegetables. Pectin gives strength to the plant tissues and assists in adherence. Pectin is a combination of different complex polysaccharides found in plants; it contains pectinic acid as major compound and is capable to build gels at appropriate conditions. Pectin is made of linear polymers of galactouronan (alpha-galacturonic acid). During the ripening process pectin's structure is altered by the enzymes which are already present in the fruits and vegetables. The enzyme which helps in the degradation of the pectin during ripening is Pectinase. These enzymes cleave the pectin chains along with the side chains attached to it, which creates the main chain. The plant tissue becomes soft in ripening as the pectin is converted from insoluble complex polysaccharides to simple soluble polymers (Prasanna V, 2009).

According to the Food and Agriculture Organization/World Health Organization, it has been stated that there should be approximately 65% of galacturonic acid in the pectin. Apart from the actual structural trait of the galacturonyl polymer i.e., the homogalacturonan, many other pectin structures are also present (Levigne *et al*., 2004).

1.1 Structure

Pectin has a molecular weight approximate 60,000–130,000 g/mol and its nature varied with extraction method and also to the kind of food. Pectin is basically a complex structure consisting of D-galacturonic acid units. The most common attribute of a pectin molecule is that it has linear chain of (1-4) linked alpha-D-galactopyranosyluronic acid moieties, making it α-D-galacturonan. It is a complex polysaccharides present in the cell wall of all the higher plants and has similarity to most other polysaccharides. It has both properties of polymole26cular and polydisperse, i.e. it is heterogeneous with respect to both chemical structure and molecular weight. The percentage and amount of individual monomeric units and their organization differ generally by the source, treatments used for isolation, and the subsequent purification techniques. Different kinds of Pectin can be manufacture by managing the sources, extraction procedures and subsequent treatments.

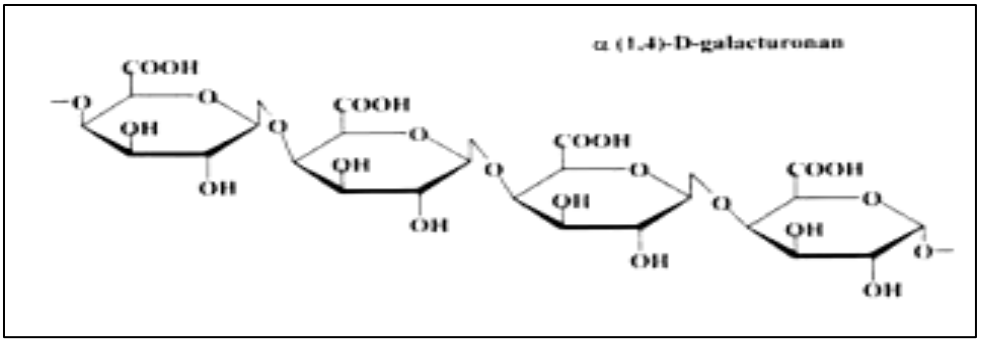

Fig.1.0 Structure of Pectin

(Sangam, P. (n.d) Essay on Cell Wall (For School and College Students)

The main part of pectin substances is composed of galacturonic acid sub-units attached by alpha (1-4) glycosidic linkage. In galacturonic acid, the carboxylic groups are partly esterified by methyl groups and fully or partly neutralized by ammonia, sodium or potassium ions. Several polysaccharides have been isolated, identified and characterized. Pectin is made from a number of polysaccharides like homo-galacturonan (HG), xylo-galaturonan (XGA), rhamno-galacturonan I and II (RG).

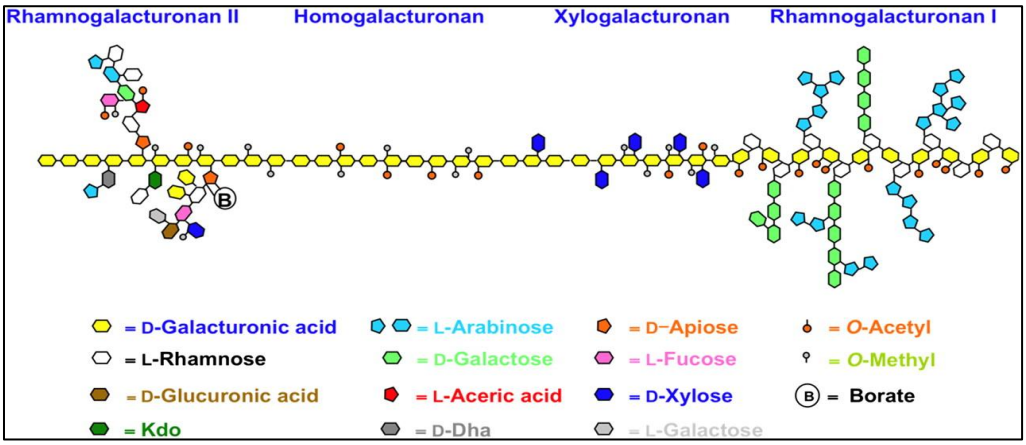

Figure 1.1 Pectin polysaccharides

(http://www.plantphysiol.org)

As shown in fig 1.1 the three pectin polysaccharides: Homogalacturonan, Rhamnogalaturonan-I and substituted galactouronans, have been isolated and distinguished from plant cell wall. Homogalacturonan has a structure of linear chains of alpha (1–4)-linked D-galacturonic acid. Other galacturonans are distinguished by the existence of saccharide units (e.g D-Xylose or D-Apiose in the type of xylogalacturonan

and apiogalacturonan) bifurcated from a main structure of D-galacturonic acid residues. Rhamnogalacturonan-I type of pectins (RG-I) have a backbone of the continuous units of disaccharide: (1-4)-α-D-galacturonic acid-(1, 2)-α-L-rhamnose. The neutral sugars that present are D-galactose, L-arabinose. Last but not the least, rhamnogalacturonan II (RG-II) is a type of pectin, which is a quite complex in its structure having highly branched structure of polysaccharide. (Sharma B. R. *et al*., 2006)

1.2 Physical properties

Pectin is soluble in water, but not soluble in aqueous solutions. Considering the solubility, the viscosity of the solution of pectin is associated to the molecular weight, DE, total volume, pH and the existence of the oppositely charged ions in the solution. The viscosity, solubility and gelation of pectin i.e., main properties of pectin which helps in increasing the gel strength or the propensity to gel, decrease solubility and increase viscosity or vice-versa. Pectins are usually consider as gelling agents, but can also behave as thickener, water binder and stabilizer. Christiaens S. *et al*., (2016) narrates enzymatic and non-enzymatic pectin changes in the span of processing of the food and process–structure–function relations. Because of its compound structure, processing of pectins may give an outcome in complex and somewhat uncertain effects on texture, viscosity and gel formation.

Commercial form of pectin is classified depending upon the methoxy content that if it is able to make gel rapidly or slowly. Pectin is divided into two classes i.e., high-methoxy pectins and low-methoxy pectins. The low methoxyl-pectins (>50% esterified) set in presence of calcium di-cation bridging. Gel strength elevates with increase in Ca^{2+} concentration but decreases with temperature and increase in the acidity level (pH < 3.0). High methoxyl pectin (<50%esterified) shows insignificant dimerization when bind with calcium because of the absence of sufficient carboxylate groups. In the presence of calcium ions and at pH (3.0 - 4.5), low methoxyl pectins form thermo reversible gels whereas high methoxyl pectins make thermally irreversible gels by utilizing enough sugars mainly sucrose and at low pH (< 3.5); the lower the methoxyl content, the slower the gel will set. The Degree of esterification was found to decrease with the help of pectin

methyl esterase, directing to increased viscosity and smooth gel in the presence of Ca^{2+} ions.

With advancement in the pectin production methods, it results in various forms; like it plays a role as nutraceutical as it has prebiotic potential and its utilization as a delivery vehicle for pro-biotics. It has been proved as a carrier for drug delivery into the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dose form. It is used as dietary fiber as well (anti-diabetic, anti-diarrheal, reduce cholesterol).

Pectin exists in all plants but the amount and constitution differs depending on the species, variety, and maturity of the plant, part of the plant, tissue of the plant, and growing condition the plant needs.

Usual levels of pectin in plants are (fresh weight) as shown below in table 1.2:

Table 1.2 Sources of Pectin

(https://www.slideshare.net/dramrhelal/stdf-pectin-presentation)

Sources	**Fresh weight (%)**
Citrus peels	30%
Apples	1-1.5%
Carrots	Approx. 1.4%
Oranges	0.5-3.5%
Cherries	0.4%

The most common sources used for pectin isolation are dried citrus peel or apple pomace; as both of them are by-products of juice manufacturing. Pectin can be extracted from these sources via dipping in warm dilute acid pH in the range of 1.5 – 3.5, the long hours of extraction involves degradation of protopectin units and result into mixture. After filtering, the extract is collected into a container and the pectin is then precipitated with the addition of ethanol or isopropanol. Alcohol-coagulated pectin is then isolated, washed with the help of ethanol and dried carefully.

1.3 *Lagenaria siceraria*: *Lagenaria siceraria* belongs to Cucurbitaceae family. It is also called as Bottle gourd. It is an annually growing plant with previous history of medicinal uses. It is grown all over India and its fruits are available in the market around the year. It is one of the magnificent fruit for human being produced by the nature having constitution of all required components that are essential for normal and good human health (Rahaman, 2003). Since it is having curative properties, and it has been employed for therapy of several ailments, which includes jaundice, diabetes, piles, hypertension, congestive cardiac failure (CCF) etc. The fruit (*Lagenaria siceraria*) is a natural source of vitamin C, β-carotene, vitamin B-complex, pectin. This plant having the low cost and annually available can be an advantage for its utilization in the preparation.

The review of literature explicitly explained the pectin role in food industries, pharmaceutical application etc. That may render it to a potential research area as a low cost gelling agent which needs thorough step by step extraction as its quantity and quality varies with the type of the source and used extraction method. So, the objectives were planned after considering the above facts, as mentioned below:

1.4 Objectives:

1. Yield calculation and process standardization via optimizing parameters in the existing methods for extraction of pectin from *Lagenaria siceraria*.
2. Quality analysis and comparison of obtained pectin for end usage.
3. Characterization and Statistical analysis of the data obtained.

CHAPTER-II

REVIEW OF LITERATURE

2.1 *Lagenaria siceraria*

2.1.1 Introduction: It belongs to the *Cucurbitaceae* family. It is commonly known as Calabash/Bottle gourd, very popular crop in India. It is in yellowish-green color having white flesh and seeds embedded in its flesh. It is available in different shapes cylindrical, round etc. It is a fast growing, annually available vegetable. Based on the usage of *Lagenaria siceraria*, it is proven that the plant species contain many bio-active compounds. (Selvaraj *et al.*, 2015)

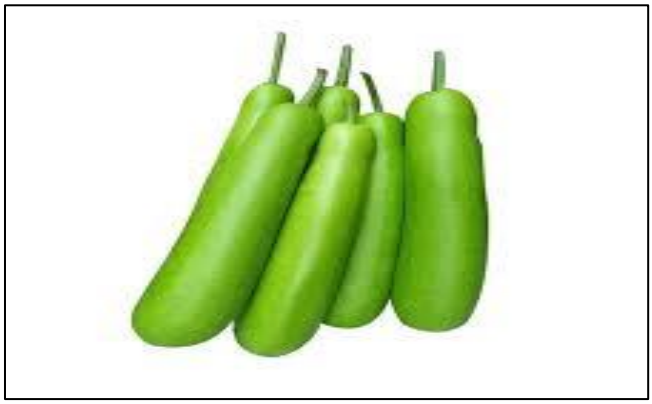

Fig.2.0 *Lagenaria siceraria* (Long-Cylindrical)

(http://freshokartz.com/fruits-vegetables/vegetables/bottle-gourd.html)

2.1.2 Physio-chemical composition of *Lagenaria siceraria*: *Lagenaria siceraria* has various components such as vitamin-B complex and ascorbic-acid along with pectin and also contains various saponins, fatty oils and alcohol (Gurpreet *et al.*, 2014). The edible fleshy portion of *Lagenaria siceraria* include carbohydrates, fats, protein and minerals, especially calcium and phosphorous. Pectin is also a fine source of β-carotene, amino acids and pectin dietary fibers (Modgil *et al.*, 2004). *Lagenaria siceraria* contains glucose, fructose and small amount of sucrose. The seed part also contains some alkaloids, carbohydrates, steroids, proteins, fats, potassium, calcium, zinc, iron, and sodium etc (Calabrese *et al.*, 1999). It contains low fat content and zero cholesterol. Its daily intake is known to reduce the blood cholesterol levels. It contains very high amount

of water content (96%) that makes it very light, easy to digest and has cooling effect on the body. It has been also seen that it cures diabetes, digestive problems, reduce liver inflammation and blood cholesterol as well. As we know, *Lagenaria siceraria* belongs to medicinal family; it helps in the treatment of many diseases. Fruit and seeds of *Lagenaria siceraria* have many medicinal values.

2.1.3 Health benefits:

- **Good source of Vitamins:** abundant source of Vitamin B complex and Vitamin C. It also contains Vitamin A and E.
- **Rich in antioxidants:** possess the property to neutralize the effect of free radicals which are produced in the body.
- **Cooling effect:** as already discussed, it contains 96% of the water contained in it gives the cooling, calming and diuretic effect on the body.
- **Low in calorie and fats:** Calories 12-14 Kcal per 100 g serving which makes it excellent source for low calories diet. It also has very less fat level content.

2.2 Pectin:

Pectins are high molecular weight heteropolysaccharide abundantly found in the kingdom of plants. Pectin acts as an elementary part of the cell wall and middle lamella of fruits and vegetables.

2.2.1 Chemical properties:

It is a complex plant polysaccharide which provides structure and firmness to the plant tissues. It is a complex polymer having backbone of galacturonic acid units joined by alpha (1-4) glycosidic linkage. In galacturonic acid, the carboxyl groups are partly esterified by methyl groups and fully/partly neutralized by ammonium, sodium or potassium ions. Pectin is responsible for gelling property and this property is dependent upon degree of methoxylation (DM). Pectins are classified depending upon their methoxy content if they form gels rapidly or slowly. Pectins are divided into two categories high methoxy pectins (more than 50% esterified pectin) and low methoxy pectins (less than 50% esterified pectin). Pectin acts as a binding material. In the unripe vegetables and fruits, insoluble pectin is bound to the cellulose microfibrills. This bonding provides rigidity to the cell wall. During ripening, the structure of the pectin is altered by some enzymes naturally present in the fruits and vegetables. These enzymes cleaves the pectin chains along with the side chains thus as a result the pectin becomes

more soluble and its grip on the surrounding cell walls gets loosened. As the result the plant tissue gets soften. (Sriamornsak P, 2009)

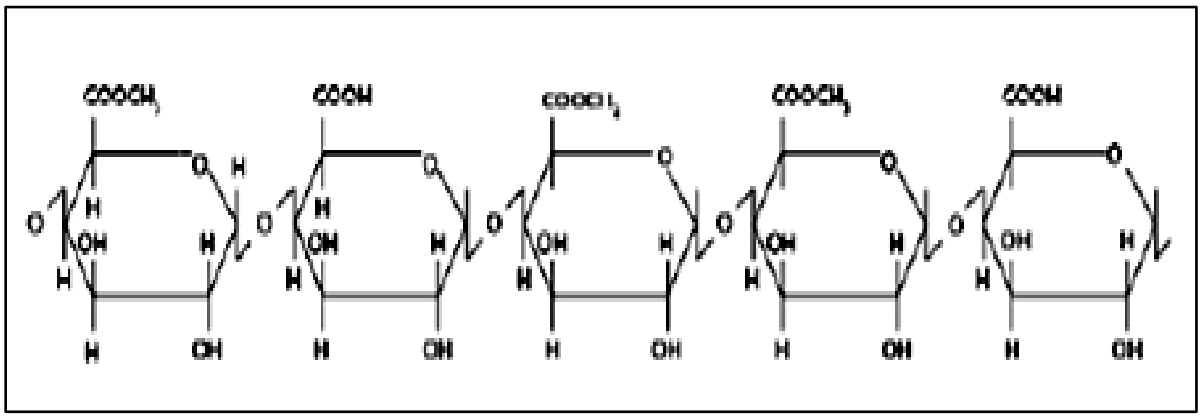

Fig.2.1 Chemical structure of Pectin

(Pawar HA, Kamat SR, Choudhary PD, 2015)

2.2.2 Types: In galacturonic acid, the carboxyl groups are slightly esterified by methyl groups, fully or partly neutralized by ammonia, sodium or potassium. Pectin is made from a number of polysaccharides like homogalaturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I and II (RG). These are generally classified into two types: Homogalacturonan and Heterogalacturonan:

2.2.2.1 Homogalacturonan: It is a linear structure polymer and referred as the smooth region of pectin. It is composed of α-D-galacturonan units connected linearly by alpha 1-4 glycosidic bonds. These can be acetylated or methyl esterified. (Sharma BR *et al.*, 2006)

2.2.2.2 Heterogalacturonan: They have further three types: (Sharma BR *et al.*, 2006)

- Xylogalacturonan: It is a α-1, 4 linked D-galacturonan chain which is changed by β-D xylose at C_3 position.
- Rhamnogalacturonan I: The D-galacturonan acid units which are present in the backbone are interrupted at various places by α-1, 2 joined L-rhamnose residues. Long galactan and arabinan chains are attached at O-4 position to these residues.

- Rhamnogalacturonan II: Cluster of complex side chains are connected at O-2 or O-3 position in the backbone of D-GA structure. (Sharma BR *et al*., 2006)

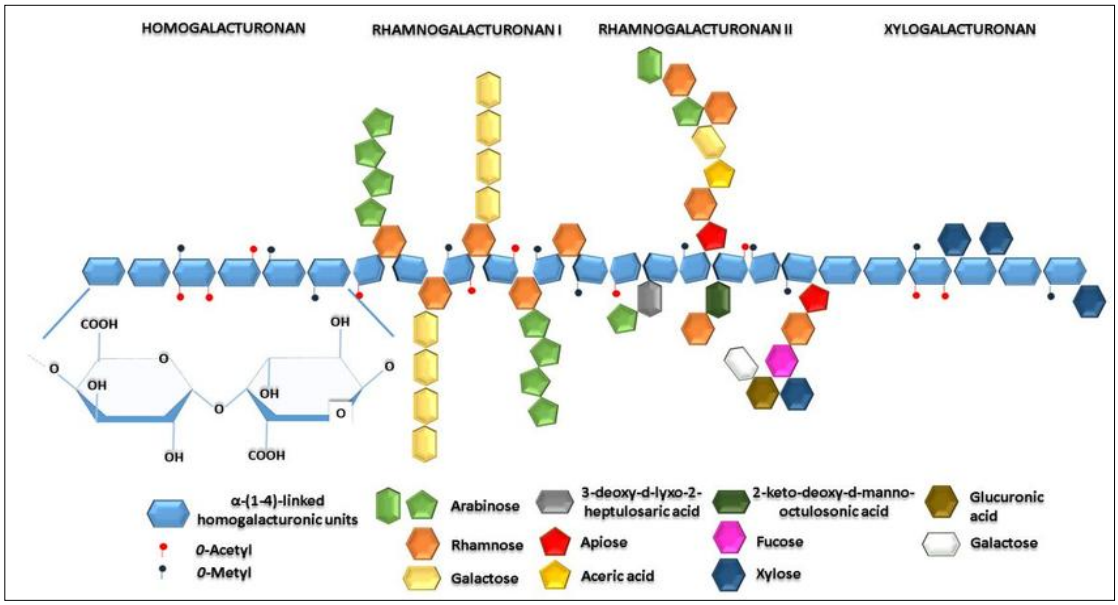

Fig.2.2 Types of Pectin

(Carneiro-da-Cunha M.G. *et al*., 2016)

An essential characteristic of pectin substance is the esterification of galacturonic acid with the acetic acid or methanol. Amount of carboxyl groups esterified with the help of methanol is called degree of methylation (DM). DM is inversely proportional to solubility. Pectin is classified into two categories depending upon the DM:

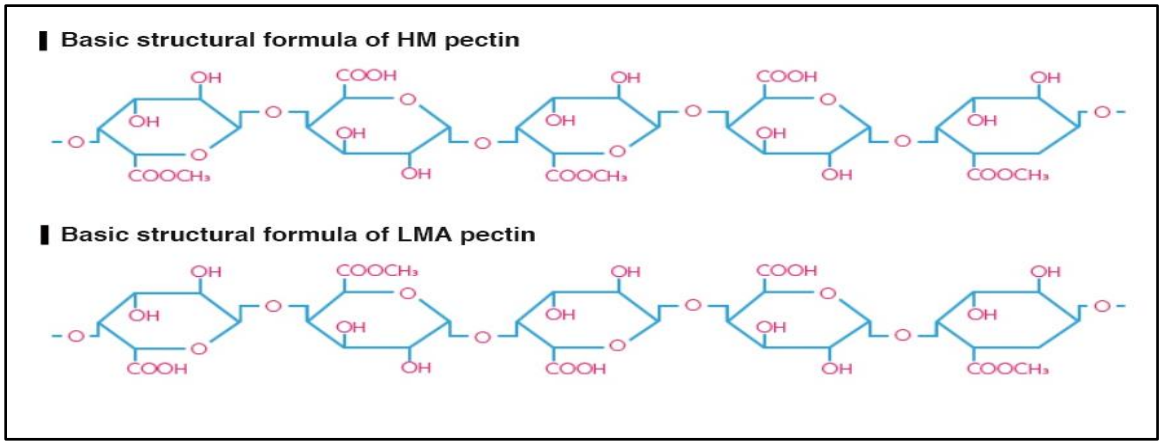

Fig. 2.3 Structures of HM & LM Pectin

(http://sansho.co.jp/en/find/polysaccharide/pectin/)

2.2.3 High methoxy (HM) pectin: More than 50 percent of carboxyl group methylated is present in the High methoxy pectin (HM). It has the ability to make gel in the presence of high sugar content and low pH value. There are no acidic groups that can gel or coagulate with calcium ions. For aggregation of pectin molecules, hydrogen bonding and hydroscopic interactions are very important. Pectin molecules in neutral or slightly acidic dispersion contain un-esterified carboxyl groups. When acid is added there is a conversion of carboxyl ions to unionized carboxyl acid group the decrease in negative charge causing less interaction between pectin and water molecules along with decrease in repulsion between pectin molecules. While with the addition of sugar a decrease in hydration of pectin observed. Thus, pectin which is in no longer is in dispersed state will not form the gel but after cooling down the less hydrated pectin forms the gel.

2.2.4 Low methoxy (LM) pectin: If the degree of methylation found less than 50 percent then they are called LM pectin. They have the capacity to form gels with the use of bivalent salts, normally Ca^{2+} ions and require low sugar content and wide pH range. The gelation of LM pectin is mainly dependent on "egg box" model.

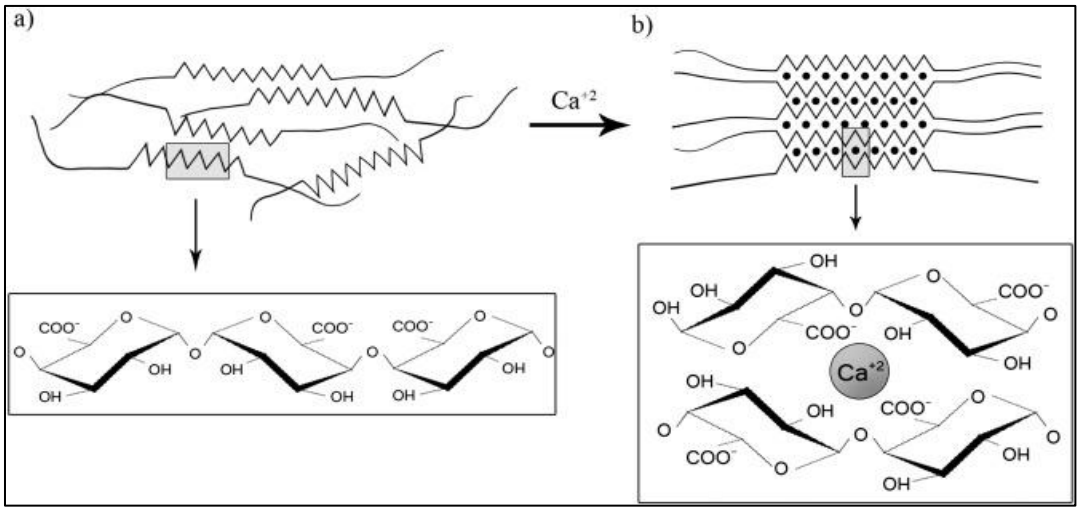

Fig.2.4 Formation of gel of LM pectin

(Oztop H.M *et al*., 2014)

According to egg box model, junction zones are created side by side linkages of galactouronan. Gal-A monomer sequence is linked intermolecular via ionic/ electrostatic bonding of carboxyl group to adjacent chains. The junction zones that are created usually consist of dimmers of helical symmetry. A number of oxygen atoms are involved in

bonding with sugar units. In the inner side of engaging chain at least seven consecutive carboxyl groups should be for the bond to be stable. Although, sugar is not needed for LM pectin to gel but if small amount of sugar is added it will lead to formation of more firm gel and there will be decrease in syneresis. Requirement of calcium decreases for formation of gel if sugar is added. But if high concentration of sugar is added, gel will not form properly (Tyagi V., *et al*, 2015).

2.2.5 Properties of Pectin powder: Powdered Pectin, when mixed in water has the capability to hydrate and form gel as it is soluble in water. Water soluble salts are mainly monovalent cations while di and trivalent cation salts are less soluble or insoluble in water. Nature of pectin in solutions is mainly Newtonian and at other moderate concentrations, it behaves as Non-Newtonian or Pseudo-plastic characteristics. The molecular weight, concentration of the preparation/sample, degree of esterification and, the pH, time, temperature and counter ions present in the solutions are all related to the viscosity of the pectin. These properties of pectin are the main criteria in the formation of the structure of cell wall of the plants. Presence of negative charges based on the determination of Degree of esterification (DE). Monovalent salts of pectin solutions showed subtle viscosity because each molecule of pectin was hydrated and independent. At lower pH, de-esterification was favored. De-esterification of HM-pectin leads to slower setting of the gel and then gradually converted into LM- pectin. (Srivastava P *et al*., 2011)

2.2.6 Characteristics of pectin:

2.2.6.1 Gelling mechanism: High Methoxy (HM) pectin has the ability to form gel when the soluble solids must be at least 55% and the pH must be less than 3.5 with sugar and acid. The gelling attribute of HM pectin increases with the increase in DE of pectin. Once the gel is formed, they are not shear reversible and thermally irreversible.

Low Methoxy (LM) pectin forms a gel with both divalent cations of calcium and magnesium. As carboxyl groups and Ca^{++} ions form junctional zones leads to the formation of coordination complex in which Ca^{++} ions are held between two pectin chains to form gel. These gels are thermally and shear reversible.

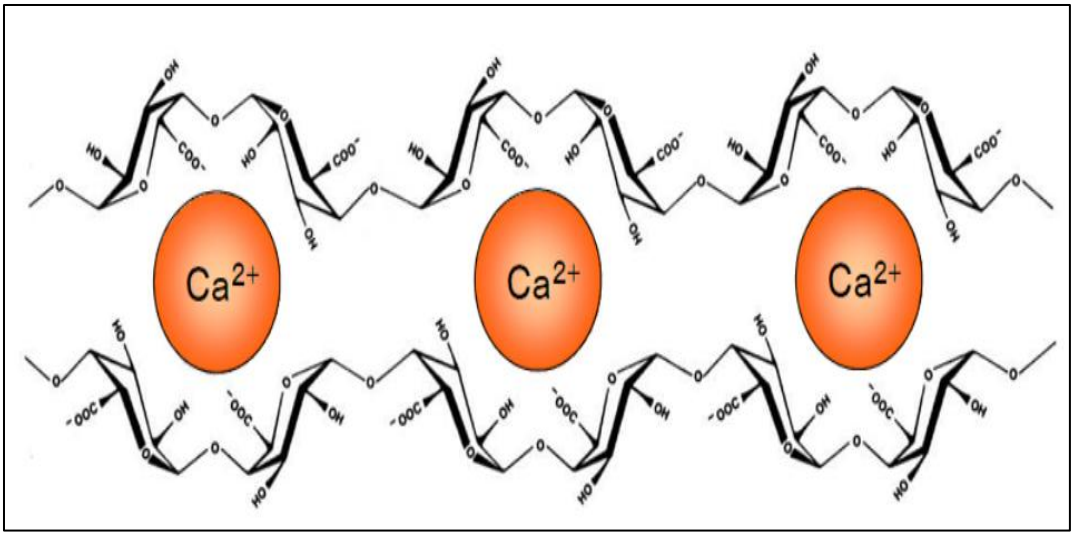

Fig.2.5 A junctional zone of LM pectin gel (Egg box model)

(sansho.co.jp/en/find/polysaccharide/pectin/)

2.2.6.2 Thickening: Relatively, Pectin showed low viscosity if compared to other hydrocolloids.

2.2.6.3 Reactivity with protein: Having the property of forming stable networks with proteins, thus HM pectin is used as a stabilizer of acidified protein drinks and desserts in order to avoid coagulation.

2.2.6.4 Acid resistance: At acidic condition, pectin shows remarkable stability compared to other hydrocolloids.

2.2.7 Pectin and its effect in jam making: In fruits and vegetables, pectin is responsible for settling/gelling characteristics of jam. Pectin substances present in middle lamella of plant tissues act as glue which holds plant tissue in place. These pectic substances have gelling ability and converted from propectin to pectin substances. The conversion of propectin to pectin is catalyzed by enzyme propectinase already present in plants. When the fruit matures propectin is converted into pectin, therefore there is increased content of pectin. When the fruits get matured, pectin is converted to pectic acid which is catalyzed by enzyme Pectinase. Among all the pectic substances, only pectin has the gelling ability so, has much interest in food industry. (Arthey D *et al*., 1995)

Pectin is an indigestible fiber, released from fruits and vegetables when boiled. In order to form a gel network these pectin molecules need to interact with each other via intermolecular interactions. But pectin molecules repulse when they are dissolved in water which means they stick to water molecules. Therefore, in order to form gel it is important to stop pectin to bind with water and start binding to each other. Jam generally sets at 104^{o}C and it is known as setting point of gel. Once it is reached, jam is allowed to cool and water is trapped in gel network (Christensen S.H *et al*., 2009).

2.2.8 Sources of Pectin: Cell walls of all the plants are formed of pectin i.e. it is a fiber which is almost present in all the green land plants may be in small amounts. Pectin is hetero-polysaccharides that make most of the composition of cell wall of fruits and vegetables. The type of fruit, its maturity or ripeness state checks the quality and quantity of pectin content. In the presence of sugar and acid, it forms jelly like mass. The soft, white spongy layer just under the colored portion of the peel is the principle source of pectin. The middle lamella of cell wall is mostly composed of pectin. Although pectin is present mostly in the plant tissues, but for the commercial production of pectin quite limited sources are present. Apple pomace and orange peel are the two main sources of commercial pectin production; both of them are left over from juice production units. Apple pomace has 10-15% (dry weight) pectin. Citrus peel relatively have higher amount of pectin i.e. 20-30. The physical appearance of commercially available pectin of citrus fruits is off white or beige in color but apple pectins are quite darker in color (Srivastava P. *et al*., 2011).

Apples, gooseberries, oranges, quince, plums, grapefruits, lemons, peaches, apricots, bananas, blackberries, raspberries have more amount of pectin whereas, soft ripened fruits like cherries, grapes, dewberries, loganberries, and strawberries have little pectin contains least amount of pectin. Substituted sources for pectin are sugar beet waste that is taken from sugar production, sunflower heads and mango waste. In vegetables, carrot is the one having the highest amount of pectin. Sweet potatoes, squash, peas, green beans and tomatoes are among the vegetable sources of pectin (Bruso J, 2017).

Table.2.2.8 Different sources of Pectin

(Colin. *D et al*., 2002)

High	*Medium*	*Low*
Cherry	Apricot	Apple
Peach	Blackberry	Blackcurrant
Pear	Loganberry	Damson
Pineapple		Gooseberry
Raspberry		Greengage
Strawberry		Guava
		Plum
		Redcurrant

2.2.9 Different extraction methods: The type of extraction method used can lead to large variations in the chemical structure of the final product. To determine the structure of extracted pectin is difficult because during processing steps of extraction/isolation from plant, storage and purification steps can lead to change in the confirmation of pectin (Novosel'skaya *et al*., 2000). The most common extraction (Acid extraction method/ Water based extraction method) conditions include optimum pH 1.5 to 3.0 by using acid Citric acid, Sulphuric acid, hydrochloric acid etc., and temperature 60^{o}C to 100^{o}C for 1 to 6 hours which varies to attain a product that has the desired gelling property and degree of methylation. The main focus is to separate the viscous material from the expanded and partly disintegrated plant source that remains a trouble (http://www.cybercolloids.net). So because of different methods designed for the extraction of pectin from different sources as described below:

2.2.9.1 Water based extraction/Acid based extraction method*:* It is the most commonly used method for isolation of pectin, which requires approximately two-three hours of boiling for obtaining a good yield of pectin. This method is based on usage diluted acid (pH up to 2.0) to isolate the pectin at temperature less than 70^{o}C. Mostly used acidifying agents are mineral acids including, sulphuric acid, citric acid, hydrochloric acid, and phosphoric acid. This extraction process runs for 3-4 hours and pectin molecules are precipitated with the use of isopropyl alcohol or ethanol. The exact extraction time differs with the source, and the type of pectin required (HM-Pectin or LM-Pectin) (Srivastava P. *et al*., 2011).

2.2.9.2 Microwave heating based extraction: It was done on a Milestone Ethos Microwave Lab Station. The solvents used were 10% ethanol, 0.05M Ethylenediamine Tetra Acetic Acid, 1M Sodium Hydroxide to get the desired pH in the range of 2.0 or nearby. Throughout microwave heating, significant pressure builds up in the inner part of the plant which changed its physical properties like disrupting the cell wall and disseminating the capillary structure of plant. Hence, the more penetration of extracting solvents inside the porous tissues improved the quality and quantity of pectin. The applicability of this extraction technique has yet to be proved over the other techniques available. This review article reveals that fifteen minutes of heating in microwave is enough to extract similar amount of pectin that was from water based extraction method after three hours of period (Srivastava P. *et al*., 2011).

2.2.9.3 **Microbial Extraction:** In this method, a microorganism was isolated which produces a protopectin-solubilizing enzyme identified as a variety of *Trichosporon penicillatum*. For isolation of pectin certain suitable conditions were determined as follows. Citrus (*Citrus unshiu*) peel was suspended in water (1:2 wt/vol.), the culture was added and fermentation proceeded over 15 hr to 20 hr at 30°C. With this method, after fermentation of citrus peel approximate 20 g to 25 g of pectin was extracted per kg of peel without maceration (Sakai T *et al*., 1980).

2.2.9.4 Enzymatic Extraction: Plant cell is composed of complex network of polysaccharides (pectin) and those enzymes which have non-pectinolytic activities have been utilized here to degrade non-pectin part of the plant. Various enzymes like cellulase, hemicellulase, polygalacturonase, protease, α-amylase, xylase, microbial mixed enzymes, celluclast, alcalase, neutrase, endopolygalacturonase and pectinesterase have been used here. With the use of cellulose enzyme leads to the highest yield of pectin. Besides that using an enzyme complex for the extraction of pectin from pumpkin has given the highest yield (14% on dry weight basis) because of the degradation of cellulose matrix of the plant cell wall. Pectin was extracted from apple and pears with the help of polygalacturonase and observed a great performance in yield around 20% and 60% higher than with chemical extraction for apple and pears respectively (Sandarani MDJC, 2017).

2.2.9.5 Subcritical water Extraction: It is a green extraction technique. This extraction method includes use of heptane/ethanol subjected to both batch/flow systems. For the flow system, sample was pressurized in the stainless steel container, and then heated at 175 ^{o}C, with the flow rate of 3 ml/min for 15 min. For batch system; sample added in pressurized stainless steel vessel with de-ionised water and heated at different temperatures ranging from 70 ^{o}C to 20 ^{o}C. Three different global varieties (Keitt, Sindhri and Kesar) were studied and the highest pectin yield (18.34%) was achieved from the Kesar variety post ethanol extraction. The degree of esterification of the extracted pectin of all the varieties was over 70% determined by both titrimetry and NMR (Matharu A. S *et al*., 2017).

2.2.9.6 Colloid Titration: It is a method which states that positive polymer ions attach stoichiometrically with negative ions in a solution which gives an end point indicating the change in color of toluidine blue and coagulation of the reactants. In this titration, the mixture of a positive polyelectrolyte like N-tri-methylated glycol chitosan mixed with any plant source extract which is having pectic acid derived from pectin by demethylation of the latter with the alkali. The pectic acid which is derived as pectin can be determined by using the values of titration and the blank experiment. The range of yield was around 0.07% and 0.1% of extracted pectin. (Okimasu S, 2014)

2.2.10 Pectin properties to be analyzed:

Pectin was extracted with the help of Acid extraction/ Water based heating method using H_2SO_4 and then purification by alcoholic precipitation. The first step after the extraction of pectin is the determination of the yield of pectin then further analysis was done.

2.2.10.1 Yield (%): Yield was calculated in order to get the net amount which is determined as the ratio of the amount of dried pectin obtained to dried material.

The percentage Pectin yield (Ypec % was determined using the equation mentioned below: {Ypec (%) = Amount of pectin extracted/ amount of initial sample} (Girma.E *et al*., 2016)

Physio-chemicals properties like Ash content, moisture content, equivalent weight, methoxy content, anhydrogalactouronic acid content and degree of esterification were to be analyzed.

2.2.10.2 Moisture content (%): It is the method used to measure the moisture percentage in the raw material. Method from Ranganna S. (1995) is used for determining moisture content:

{Moisture%=W1*W2/W1-W}

Where, W1 is weight of petridish with sample, W2 is the weight of the petridish with dried sample and W is the weight of the sample (Ranganna S. (1995).

2.2.10.3 Ash content (%): It is the measure of the total amount of minerals present in it. The ash content was determined by adopting the method of (Ranganna S., 1995).

Percentage of ash was calculated from the formula:

{Ash% = W1-W2/W}

Where, W1 is final weight of the dish with ash, W2 is the weight of the dish and W is the weight of the sample (Ranganna S. (1995).

2.2.10.4 Total Solids (TS) (%): The TS of the pectin sample was determined by subtracting the percentage of moisture content from hundred as followed by (AOAC, 2000). It is expressed in the following expression:

{Total solids (%) = 100-Moisture content (%)}

2.2.10.5 Equivalent weight: Equivalent weight of extracted pectin can be defined as the total number of free galacturonic residues (non-esterified) present in the chain of pectin.

Equivalent weight was obtained by tit rating a known amount of the pectin against standardized NaOH solution which is 0.1N and gives faint pink color end point in the presence of Phenolphthalein indicator upon reaction. It can be calculated with this formula:

{Equivalent weight= Wt. of the sample*100/Titer value*Normality of the alkali}(Girma E. *et al*., 2016)

2.2.10.6 Methoxy content: Determination of methoxy content in pectin is done by the saponification and then titration of the liberated carboxyl group against standardized

solution of NaOH (0.1N) using phenol red indicator giving faint pink color end point. Methoxy content can be calculated with the help of an equation:

{Methoxy content (%) = {(Titer X Normality of NaOH X 31) X 100) / (Weight of the sample X 1000}

Where, 31 is the molecular weight of methoxyl (CH_3O) (Girma E. *et al*., 2016)

2.2.10.7 Total anhydrouronic acid content: It helps in the determination of the purity and degree of esterification, and to determine the physical attributes. Values of equivalent weight and methoxy content are used to calculate the content of Anhydrouronic acid:

{Total Anhydrouronic acid content (AUA %) = {(76 X 0.1z X 100) / (WX100+176 X 0.1y X 100) / W) X 100}

Where, molecular weight of AUA (1 unit) =176g, z= ml titer value of NaOH from equivalent weight determination, y= ml titer value of NaOH from methoxy content determination, W=weight of the sample (Girma E. *et al*., 2016)

2.2.10.8 Degree of Esterification: It is the proportion of esterified galacturonic acid groups to the galacturonic acid groups present. The degree of esterification is determined from methoxy content and anhydrouronic acid content using the equation described below: {Degree of Esterification= (176 X Methoxy content (%) X 100) / (31 X AUA) (%)} Where, AUA (%) is the percentage of Total Anhydrouronic acid content and 176 is the molecular weight of AUA (1 unit) (Girma E. *et al*., 2016).

2.2.10.9 Fourier Transform Infrared Spectroscopy (FTIR): Fourier Transform Infrared Spectroscopy, also known as FTIR Spectroscopy. It is a method which is helpful to determine some chemicals which are inorganic or organic and sometimes polymeric materials. It is also useful in analyzing some unknown components from a mixture. It is an analytical technique which refers to a development of the system in the manner in which the information of input is collected and then changed from an interference pattern to a spectrum. It is a glorious tool for determining the types of chemical bonds that are present in a component by producing a spectrum which is like a molecular 'fingerprinting". Degree of esterification of pectin was determined by FTIR spectroscopy. It also helps in finding the functional groups that are present in the Pectin (Kyomugasho C. *et al*., 2017).

Pectin DE can be evaluated using the peak area relation of the free carboxyl groups (1650 cm-1) and esterified groups (1750 cm-1) (Gnanasambandan *et al.*, 2000). Pectin esterification degree was determined by using the peak areas values of the bands using the expression mentioned below:

{DE = Area of esterified carboxyl groups/ (Area of esterified carboxyl groups + Area of non-esterified carboxyl groups) × 100}

2.2.10.10 X-Ray diffraction (XRD): The properties of a component having an arrangement of atoms in its crystal structure. The atomic region of a crystal let an incident beam of X-rays to interfere with one another as they leave the crystal. This process is called X-ray diffraction. X-ray diffractograms showed that physical and chemical changes along with the tri-dimensional network are not due to cross-linking process. The location of diffractions maximum can be correlated to the magnetite pattern. There is presence of broad lines in the XRD patterns having different reasons for it. The content of magnetite can give an idea about the peaks symmetry and broadening of the peaks in the XRD pattern. Second reason for the broadening of the lines can be due to the small size of the particles in the XRD. (Nataliya *et al*., 2014)

2.2.11 Pectin like similar products available in the market: Pectin is mainly derived from plants. It gives strength and it is the main element of the structure of the cell wall of fruits and vegetables. It is a natural additive that is extracted from fruits and vegetables. It has benefits in food and some in pharmaceutical industries as well. But there are some other alternatives of pectin present in the market.

2.2.11.1 Gelatin: Gelatin is derived from collagen. It is a substance that is mainly composed of amino acids. Glycine and proline are the main amino acids contribute to gelatin structure. Gelatin is mainly obtained from bones, fibrous tissues and organs of animals.

2.2.11.2 Carrageen: Carrageen is also known as Irish moss which is seaweed. It is used when to make softer gels and puddings. They have the tendency to form different gels at room temperature.

2.2.11.3 Agar-Agar: It is a flavorless gelling agent derived from algae. It is also known as Kanten. Agar-Agar is the most common alternative to gelatin for vegetarians. It is available in the form of flakes and powder in the market.

2.2.11.4 Vegan gel powder: It is a substitute of Gelatin. It is known as vegetarian gelatin. It is composed of vegetable gum, adipic acid and tapioca dextrin (www.peta.org/food/gelatin-alternatives/).

2.2.12 Applications: Pectin has a relative high molecular mass and so can be transformed into hydrogels, intended into versatile network of polymer units that can inflate but don't disperse in water. The solubility of pectin can only be possible at low pH with varied concentration of acid that eases the development of coil entanglements and as such forms the gel. The mechanism of gel forming and development of hydrogels from pectin has grabbed the attention of manufacturers of biomedical engineering for medical applications like drug delivery, tissue engineering and wound healing. Pectin has been used widely as technical adjuvant and had fully exploited its structural miscellany. Several diverse conformations of pectin have lead to distinct gelling ability, emulsion, thickening property, emulsion stabilities, and thereby release affects in complex food matrices. In pharmaceutical industries, pectin is utilized as an expedient because of its non-toxicity, low production costs, and gelling activity properties. In the digestive tract with the help of bacteria it is completely converted to its simpler sugars without ill effects. Hence, it helps in gradually slower emptying of the stomach, which leads to the decrease in blood sugar level after eating.

Pectin in association with other polysaccharides innovatively used in making edible coating like alginate and chitosan have been made to assess the storage ability on food and fruits. Pectin being an agricultural by-product is used as source of prebiotics known as pectic oligosaccharides. The rise in the utilization of pectin in delivering of drug has been made possible by the physiochemical attributes of pectin like muco-adhesiveness, ease of solubility in basic conditions, resistance of deterioration by protease & amylase of the upper gastrointestinal tract and the capability to make gels in acid conditions which made this polysaccharide to target diverse drug delivery formulations. In addition to food and pharmaceuticals, recent studies reported that pectins exhibit immune-modulating

properties. In tissue engineering applications like bone cell culture, in wound healing applications for binding active drugs or growth factors and protecting against bacteria was made possible because of the hydrogels from Pectin. (Carneiro-da-Cunha *et al*., 2016)

2.2.12.1 Application in Pharmaceutical industry

It has an important application in Pharmaceutical industry. Intake of at least 6g/day of pectin has a significant effect in cholesterol reduction. It has been reported that 13% of the decrease in serum cholesterol within two weeks of therapy (Miettinen TA, Tarpila S, 1977). It has been used against poisoning as it behaves as a natural prophylactic material. It has been reported that it was used in eliminating lead and mercury from the respiratory and gastrointestinal tract (Kohn, 1982). It has been reported that pectin helps in lowering down the rate of absorption of food which means it reduces the rate of breaking down of the food by immobilizing the food ingredients in the intestine. Reduction in the uptake of the food is due to hindrance in the contact between the intestinal enzymes and the food because of the impact of thickness of pectin layer which leads to the lesser absorption of glucose or so other nutrients (Flourie D *et al*., 1984). Pectin initiates quick satiety because of its large water binding capacity that leads to the decrease in hunger and as such reduction in the food consumption. Systematic drug release is designed in the colon as such that the drug should remain protected during the transition through the stomach and small intestine. Pectin has been reported as a drug delivery system in colon (Liu L.S *et al*., 2003). The Fishman and coffin, 2004 studied that the pectin is a film-forming polymer. In pharmaceutical and food industries, these film forming polymers were used in coatings and controlled release carriers.

Pectin has been used for oral drug delivery which has controlled release system as reported by some authors enlisted below:

Table.2.2.12 Controlled release formulations using Pectin

(Sriamornsak P, 2003)

Dose form	Types of pectin	Applications	References
Coated pellets	Low methoxy pectin (amidated and non-amidated)	With the help of interfacial complexation, insoluble calcium pectinate gel is prepared for sustained delivery.	Sriamornsak P *et al*., 1997
Gel beads	Low Methoxy pectin	Pectin beads were prepared by ionotropic gelatin. To generate a highly cross linked structure ionotropic gelation is a technique which involves interaction of cation (or an anion) with an ionic polymer.	Aydin *et al*., 1996
Microspheres	Low Methoxy pectin	With the help of emulsification technique pectin-based microspheres were prepared.	Wong *et al*.,2002
Particulates	Low Methoxy pectin	Particulates are prepared with the help of Alginate, Polylysine and Pectin.	Liu and Krishan, 1999
Tablets	Pure and standardized pectin	Binding agents and delayed drug release.	Slany *et al*.,1981
Tablets	High Methoxypectin	Hydrogels matrix system for the incorporation of drugs.	Sungthongjeen *et al*.,1999

2.2.1.12.2 Applications in Food industry

Generally, in jellies and jams pectin has been utilized as a gelling agent. Due to variation in the degree of esterification, that created a large variety of pectin. It has been used in many foods as a thickener, stabilizer, and emulsifier (FAO, 2009).

It is being used in Dairy industry because of its stabilizing and thickening properties. To avoid precipitation and agglutination of caseins, pectin has been utilized in low acid milk having pH in the range of 3.5 to 4.5 (Voragen *et al*., 1995). Pectin has been used differently in yoghurt which depends upon the characteristics of the product; as a thickener, water binder in stirred yoghurt and as an emulsifier to give fat like taste and texture in fat free yoghurt.

Pectin also has applications in bakery industry. It helps to retain the moisture, and to increase the volume, gives flexibility and softness in breads. The stabilizing and thickening property made its usage in variety of other food products as well such as mayonnaise, tomato ketchup, salad dressing, beverages and protein foams (Pilnik and Voragen, 1992).

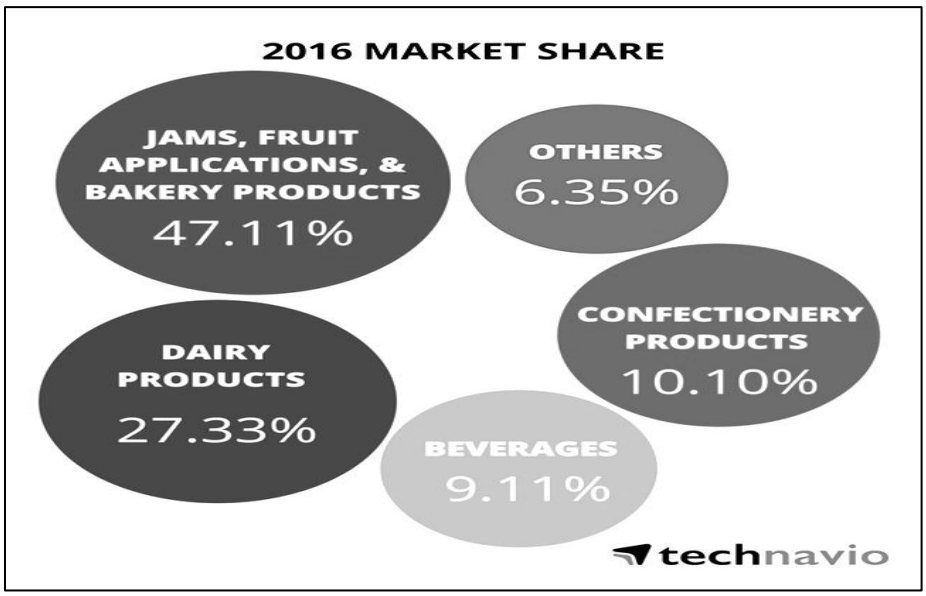

Fig.2.6 Applications of pectin in global market of food industry (2016)

(https://www.businesswire.com/news/home/20170306005571/en/Global-Pectin-Market-Driven-High-Demand-Generated)

CHAPTER-III

MATERIALS

3.1 Pre-requisites for the study

3.1.1 Collection of the *Lagenaria siceraria*: The *Lagenaria siceraria* was obtained from the vegetable vendors from the local market near TIET campus, Patiala. The cylindrical one was chosen for the study.

3.1.2 Weighing balance: Electronic weighing balance was used for measuring *Lagenaria siceraria* powder and other chemicals.

3.1.3 Muslin cloth: Muslin cloth with pore size 7-22mm was used in the filtration step provided by the BTD, TIET.

3.1.4 Refrigerator (LG): Refrigerator was used for storage of *Lagenaria siceraria* which is kept in STEP a part of Biotechnology Department, TIET, Patiala.

3.1.5 Mixer grinder (Bajaj): Mixer grinder helps in grinding to make the powder of *Lagenaria siceraria* which is present in STEP.

3.1.6 Steamer (Pristine): Steam was created by steamer to give steam blanch to *Lagenaria siceraria* which is kept in STEP.

3.1.7 Aluminium foil (Fresh wrap): Aluminium foil was used for covering while heating in water bath. It was provided by DBT department, TIET, Patiala

3.1.8 Water bath: It is required to optimize the temperature for extraction of pectin from *Lagenaria siceraria at* STEP.

3.1.9 Filter paper: For the filtration of coagulated pectin from the mixture of ethanol and juice of *Lagenaria siceraria*. These were provided by DBT, TIET.

3.1.10 Digital thermometer: It was used to measure the temperature of the acidified slurry in water bath. Digital thermometer was provided by the project guide.

3.1.11Digital pH meter: Digital pH tutor was used to measure pH of the *Lagenaria siceraria* juices. It was provided by the project guide.

3.1.12 Glass bottles (Jars): These were used to store the chemical solutions. It was provided from DBT, TIET.

3.1.13 Autoclave: Autoclave was used to sterilize the glass wares which were used in the work. It was kept in STEP, TIET.

3.1.14 Centrifuge (Micro Hematocrit Centrifuge Model 3220): It was used for the centrifugation. It was kept in STEP, TIET.

3.1.15: Desiccator: It was used to keep the hygroscopic sample free from moisture. It was provided in the STEP, TIET.

3.1.16 Muffle furnace (Thermo Scientific CP1065058 Programmable Muffle Furnace, 240VAC): It was used for determining the Ash content. It was provided by the DBT, TIET.

3.1.17 Hot air oven (Steri Techno Fab): It was used for the drying of the sample under suitable conditions. It was provided in the STEP, TIET.

3.1.18: Incubator (Excella-E25R-Economical-Console-Incubator-Shaker): It was used for the incubation and drying at moderate temperature. It was kept in STEP, TIET.

3.1.19 Lyophilizer (FD-18 series Freeze Dryer): It was used to lyophilize the sample. It was kept in TIFAC CORE, TIET.

3.1.20 Cold room: It was used to store the sample. It was kept in DBT, TIET.

3.1.21 Reagents: Ethanol (96% & 70%), H_2SO_4 (1N), NaCl, Phenol red indicator, Distilled water, NaOH (0.1N & 0.25N), HCL (0.25N) etc.

3.1.22 Glass wares: Beakers, Conical flasks, Dropper, Glass rod, Spatula, Tarson tubes, Measuring cylinder, Burette, Petri plates (Glass), Trays, Vessel, Conical filter etc. all were made available by DBT, TIET, Patiala.

All of these resources were provided by DBT, TIET.

CHAPTER-IV

METHODS

4.1 Analyzing the percentage yield of pectin in *Lagenaria siceraria*

4.1.1 Sample preparation

- *Lagenaria siceraria* was bought from the local market.
- It was washed properly then chopped into smaller pieces, blanched and cooled finally.
- After cooling, it was kept in the hot air oven overnight for drying.
- After drying, fine powder was made with the help of grinder and then it was kept in airtight container for further analysis.

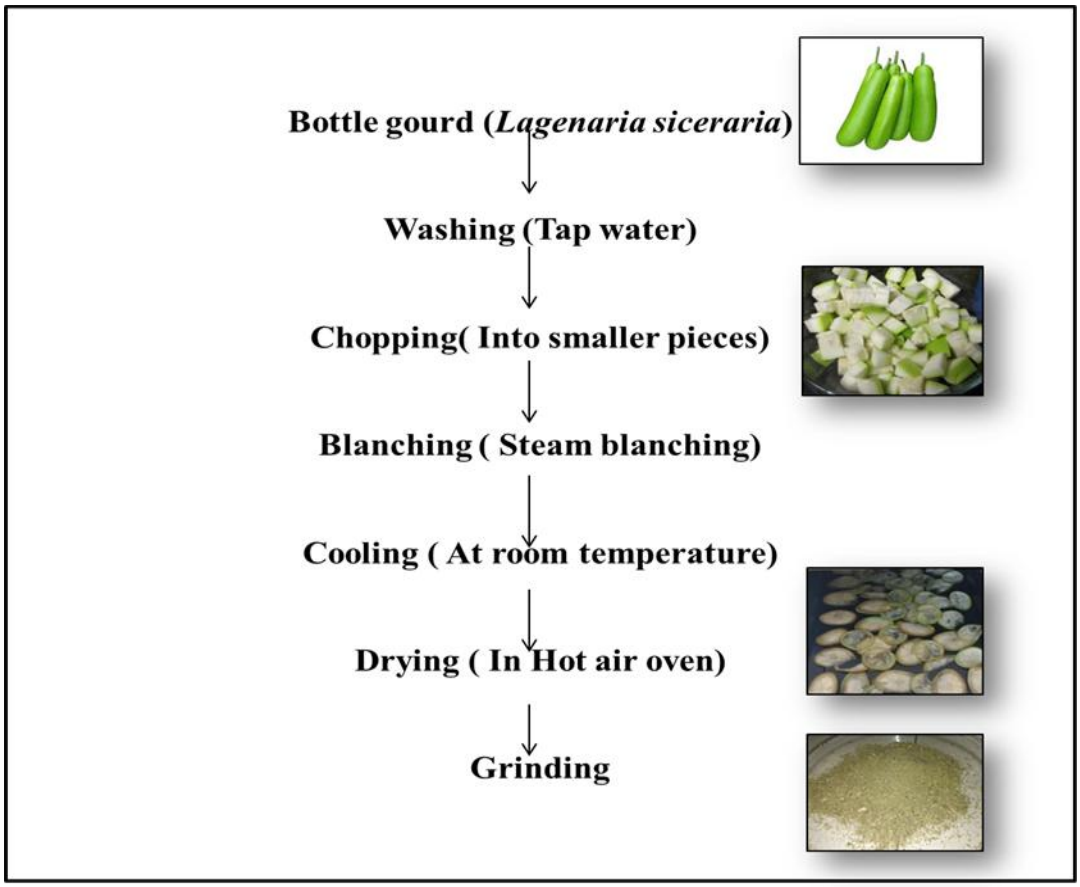

Fig. 4.0 Flowchart of methodology of sample preparation

4.1.2 Extraction process

- Acid extraction was done with the help of H_2SO_4 to maintain the pH-2.0 in *Lagenaria siceraria* powder and distilled water mixture.
- It was kept in shaking water bath for 2 hours at 60^{o}C and then cooled at room temperature.
- Filtration was done with the use of muslin cloth then filtrate was collected and precipitated with the help of 96% ethanol.
- Precipitated filtrate was kept for 3 hours at room temperature & then skimmed off.
- Washing was done 2-3 times with 70% ethanol; washed pectin was then dried in hot air oven, grinded and used for further analysis.

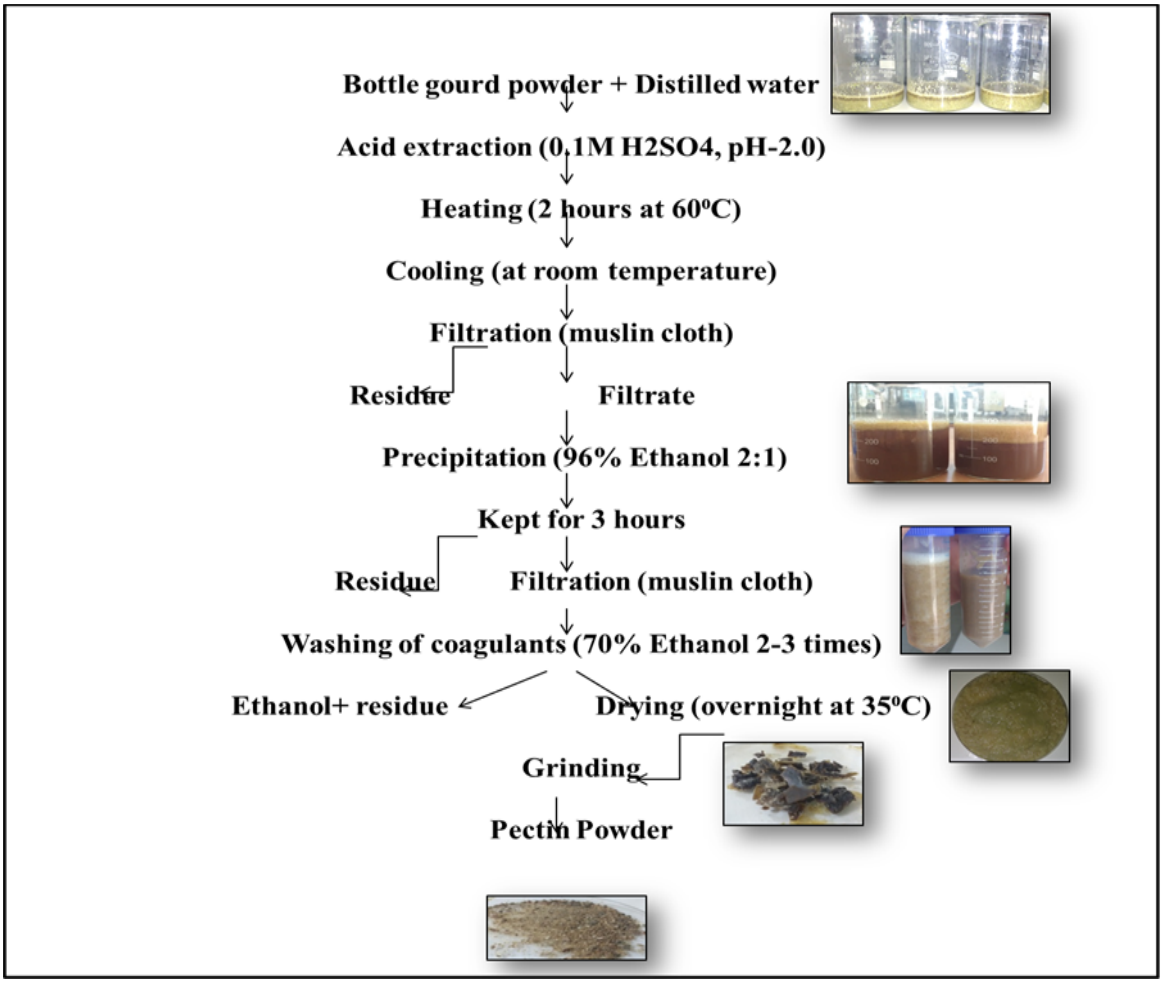

Fig. 4.1 Flowchart of extraction process

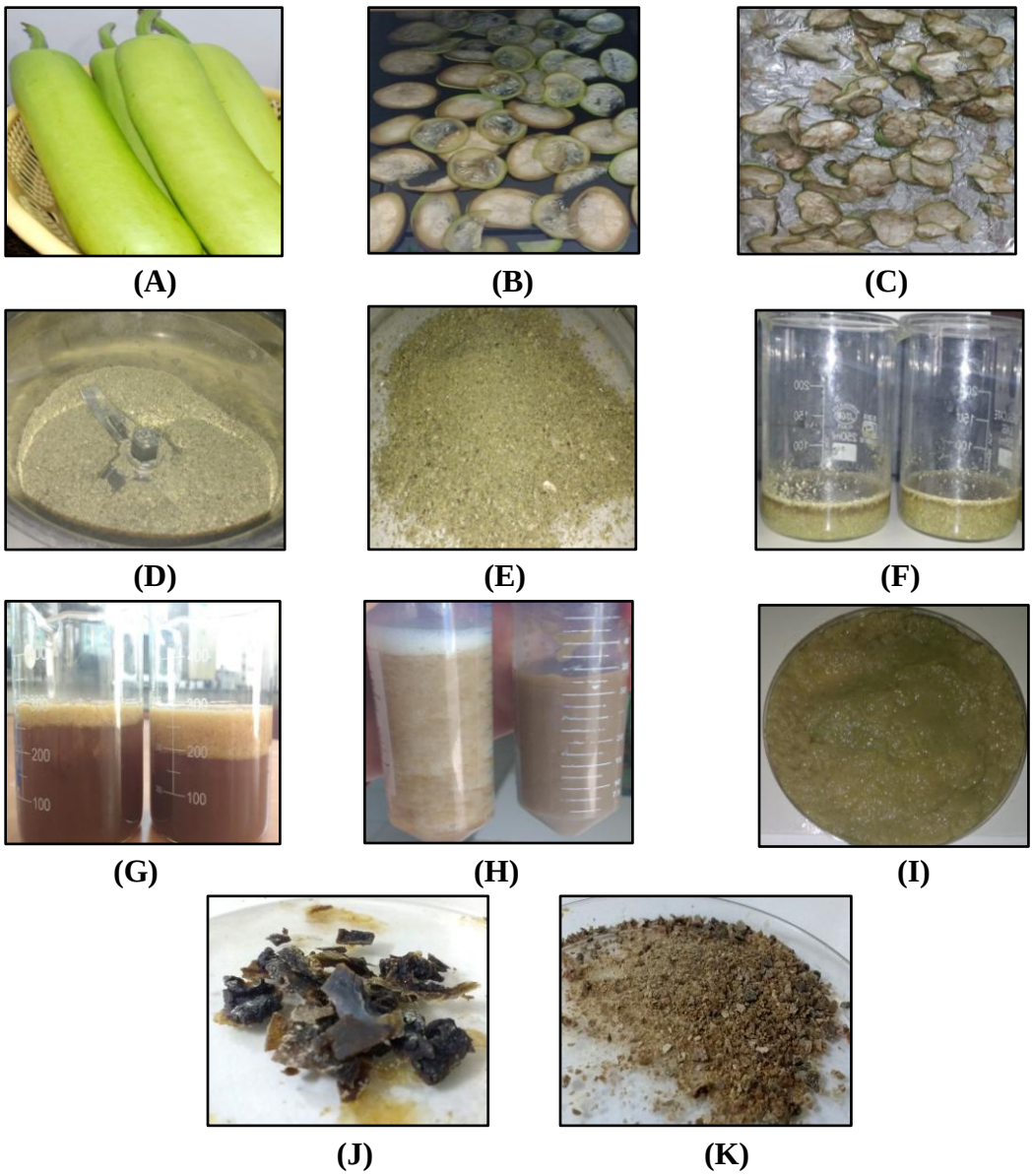

Fig. 4.2 Graphical representation of extraction methodology of *Lagenaria siceraria* pectin

(A) Raw *Lagenaria siceraria*, (B) Cut pieces of *Lagenaria siceraria*, (C) Dried pieces of *Lagenaria siceraria*, (D) Grinding of dried prices of *Lagenaria siceraria*, (E) Powder of *Lagenaria siceraria*, (F) Powder of *Lagenaria siceraria* in Distilled water, (G) Acid extraction, (H) Washing of coagulants with Ethanol, (I) Pectin before drying, (J) Pectin after drying, (K) Pectin powder

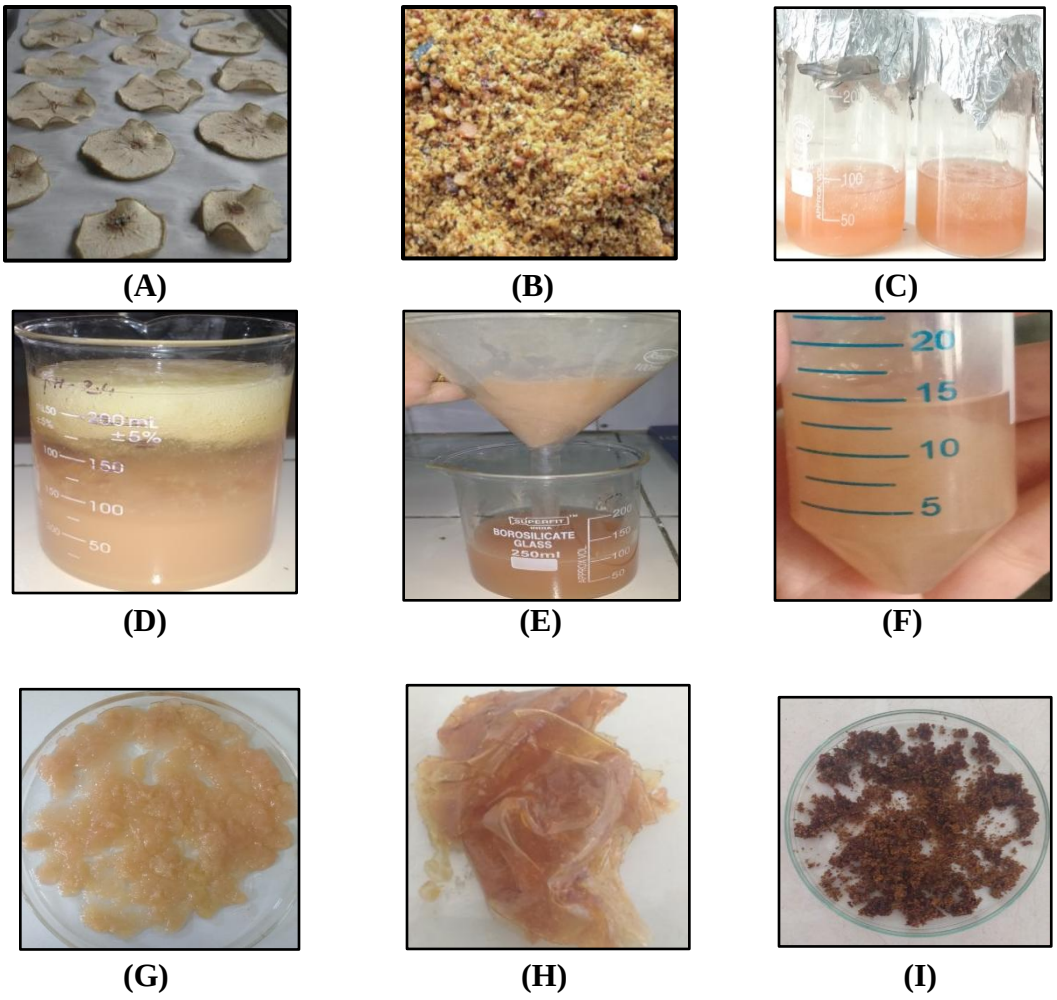

Fig. 4.3 Graphical representation of extraction methodology of Apple pectin

(A) Dried apple pieces, (B) Apple powder after grinding, (C) Apple powder with distilled water, (D) Acid extraction of Apple powder, (E) Filtration, (F) Washing of coagulants, (G) Apple pectin before drying, (H) Apple pectin after drying, (I) Apple pectin powder

4.2 Quality analysis of extracted Pectin from *Lagenaria siceraria*:

4.2.1 Yield (%): It was determined by considering the weight of the powder before and after the extraction. The percentage of pectin yield (Ypec %) was determined from the following equation:

{Ypec (%) = Amount of pectin extracted/ Amount of initial sample} (Girma.E *et al*., 2016)

4.2.2 Moisture content (%): It was evaluated by using the oven drying method (AOAC, 2000). In order to determine the moisture content of extracted pectin, first weigh the empty petridish (W). Then the sample was kept in the clean petridish and weighed before drying (W1). Petridish containing the pectin was kept in Hot air oven at 105-110^{o}C for 3 hours. Once the pectin sample gets dried after 3 hours, it was cooled, kept in desiccator and then containing the pectin was reweighed. Moisture content was calculated with the help of expression: {Moisture (%) = (W1-W2/W1-W)*100}

Where, W1 is weight of petridish with sample, W2 is the weight of the petridish with dried sample and W is the weight of the sample.

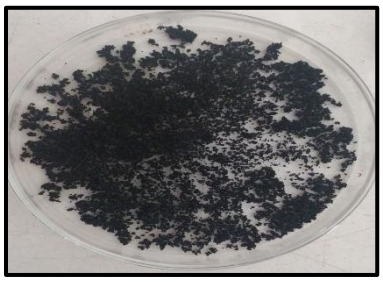

Fig. 4.4 Moisture content (%) of *Lagenaria siceraria* pectin

4.2.3 Ash content (%): Ash content was evaluated by charring the oven dried sample and then placing the sample in a pre-weighed crucible and after charring kept in muffle furnace at 440-750^{o}C for 4 to 5 hours. After cooling the crucible was weighed. The percentage of ash content was evaluated by using the method of (Ranganna S., 1995). Ash content was calculated with the help of following formula: {Ash% = W1-W2/W}

Where, W1 is final weight of the dish with ash, W2 is the weight of the dish and W is the weight of the sample.

Fig. 4.5 Ash content (%) of *Lagenaria siceraria* pectin

4.2.4 Total solids (TS): The TS of the pectin sample was determined by subtracting the percentage of moisture content from hundred as followed by (AOAC, 2000). It is expressed in the following expression: {Total solids (%) = 100-Moisture content (%)}

4.2.5 Equivalent weight: It is calculated by titration with sodium hydroxide (NaOH; pH-7.5) with phenol red solution. It is also used to evaluate the total anhydrouronic acid content and degree of esterification.

Pectin sample was weighed (0.05g) in a 250ml conical flask and it was moistened with 0.5ml of ethanol (70%). Then 1g of sodium chloride was mixed in the above solution followed by 10ml of distilled water and 3-6 drops of phenol red indicator. Titration was done with 0.1N Sodium hydroxide (NaOH) until the color of the mixture turned in to pink at the end point. The change in color was persistent to at least 30 seconds. Equivalent weight calculated with the help of following expression:

{Equivalent weight= (Wt. of the sample/ Titer volume of alkali (ml)*Normality of alkali)*1000}

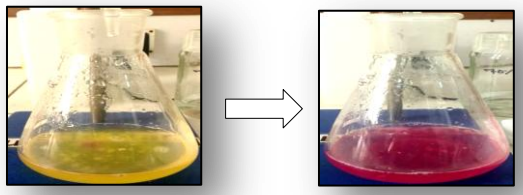

Fig.4.6 Equivalent weight (When titrated with 0.1N NaOH the color turned from yellow to pink)

4.2.6 Methoxy content: It helps in controlling the gel setting time of the pectin, the reactivity to polyvalent cation and their utilization in the development of low solid gels and fiber.

Methoxy content was estimated using the neutralized mixture that was obtained during the equivalent weight determination. In this neutralized solution, 0.05g of pectin added with 25ml of 0.25N of NaOH. This solution is mixed properly and then allowed to stand for 30 minutes at room temperature. After the 30 minutes incubation, 25ml of 0.25N of

HCL was added and titrated against 0.1N NaOH to the same end point as before in the equivalent weight determination.

The percentage amount of methoxy content is calculated using the following equation:

{Methoxy content (%) = Titer volume*Normality of alkali*31*100/wt. of sample (mg)*1000}

Where, 31 is the molecular weight of the methoxy (CH_3O).

4.2.7Anhydrouronic acid content: It is used to check the purity and degree of esterification of the pectin. By using the values of equivalent weight and methoxy content, the percentage of anhydrouronic acid content can be calculated with the following equation:

{Total Anhydrouronic acid content (AUA %) = 176*0.1z*100 /W*100+ 176*0.1y*100 /W*100}

Where, molecular weight of AUA (1 unit) =176g, z= ml titer value of NaOH from equivalent weight determination, y= ml titer value of NaOH from methoxy content determination, W=weight of the sample (Girma E. *et al.*, 2016)

4.2.8 Degree of Esterification: It is the proportion of esterified galacturonic acid groups to the galacturonic acid groups present. It is determined by using the values of methoxy content and anhydrouronic acid content using the following equation:

{Degree of Esterification= 176*Methoxy content (%)*100/31*AUA (%)}

Where, AUA (%) is the percentage of Total Anhydrouronic acid content and 176 is the molecular weight of AUA (1 unit).

4.3 Statistical analysis: Multi-variance analysis (ANOVA) was used to analyze the optimum conditions in relation to the acid used for pectin extraction. ANOVA (Analysis of Variance) was performed to check that is there any significant difference between the readings of the parameters of different quantitative properties. The data obtained for each functional property was subjected to determine the level of significance ($P<5\%$).

4.4 Characterization of the extracted Pectin:

4.4.1 Fourier Transform infrared spectroscopy (FTIR): FTIR spectra of lyophilized powdered form of pectin samples of *Lagenaria siceraria* and apple were recorded using FTIR Spectrophotometer in the range of 400-4000 cm^{-1} for the analysis of degree of esterification. It was done in SAIF labs in Punjab University, Chandigarh.

4.4.2 X-Ray diffraction (XRD): The X-Ray diffraction of pectin samples of *Lagenaria siceraria* and Apple were performed on X-Ray diffractometer. It was done in CIL in Punjab University, Chandigarh. The intensity of the diffracted X-rays was measured as a function of the diffracted angle 2θ with the help of copper (Cu) as an anode material.

These were some parameters analyzed to check the quality of the pectin obtained from the *Lagenaria sciceraria* to the control sample that is apple pectin. To analyze the obtained sample is pectin or so.

CHAPTER-V

Results and Discussions:

In the present study, the pectin from *Lagenaria siceraria* has been extracted with the help of acid extraction method using 0.1 M of H_2SO_4 along with the optimization of different parameters which include pH which was set at 1.5, 2.0 and 2.5 values. Beyond, this time and temperature of treatment with acid was optimized to obtain highest yield of Pectin. Time was varied at 1hr, 2hrs and 3hrs while the temperature changes from 50 ^oC, 60 ^oC and 70 ^oC respectively. After optimization of the parameters, the extracted pectin sample from *Lagenaria siceraria* was analyzed for percentage yield and its quality was analyzed through parameters and were observed as Moisture content of 14.53% (pH-1.5), 16.78% (pH-2.0) & 19.91% (pH-2.5), Ash content of 7.75% (pH-1.5), 10.83% (pH-2.0) & 14.20% (pH-2.5), equivalent weight of (*Lagenaria siceraria* pectin-9.7g/mol Apple pectin-2.3g/mol Standard pectin-0.5g/mol, methoxyl content (*Lagenaria siceraria* pectin-90.8% Apple pectin-1.4% & Standard pectin-1.6%), total anhydrouronic acid content (*Lagenaria siceraria* pectin-1222.5%, Apple pectin-1525.3% & Standard pectin-1391.6%) and degree of esterification (*Lagenaria siceraria* pectin-17.9%, Apple pectin-5.6% & Standard pectin-8.9%). For the characterization of the extracted pectin sample FTIR and XRD analysis was done in SAIF & CIL, Punjab University, Chandigarh. The pectin samples were analyzed quarter times for each parameter to achieve the best results. The experimental data obtained from all the parameters has been statistically analyzed with the help of suitable software. The whole work has been done in Science and Technology Entrepreneur's Park (STEP), TIET, Patiala and the results are discussed in the following headings and subheadings.

5.1 Standardization of Pectin from *Lagenaria siceraria*:

5.1.1 Quantification of *Lagenaria siceraria* powder: *Lagenaria siceraria* was bought from the market around 2kg and washed, peeled, chopped, blanched, dried and then grinded to powder form for further use. After grinding, the powder of *Lagenaria siceraria* was weighed that came approximately 284g. For further analysis, the *Lagenaria siceraria* powder yield (284g) was divided into three replicates i.e. 90g each.

To calculate the percentage yield, the weight of the whole sample and then the powder was taken.

The percentage yield (Y %) was determined with the following expression:

{Y (%) = Weight of *Lagenaria siceraria* powder/ weight of the *Lagenaria siceraria**100}

{Y (%) = 14.2%}

5.1.2 Percentage yield of *Lagenaria siceraria* Pectin powder considering different parameters:

Different parameters that have been considered for the optimization to increase the yield of pectin powder from the *Lagenaria siceraria* were the pH, temperature and time.

Table 5.1.2 Effect of various parameters (pH, Temperature and Time) on percentage yield of pectin extracted from *Lagenaria siceraria*

Parameters			Yield (%)	Mean (%)	SD (%)
pH	**Temperature (^{o}C)**	**Time (min)**			
1.5	50	60	3.81	3.84	0.026
2.0	60	90	3.86		
2.5	70	120	3.85		

As the values shown in the table 5.1.2 the change observed majorly because of variation in pH rather than time and temperature. The average values came out to be like 3.81% @ pH 1.5, for 60min and 50^{o}C, 3.86%@ pH 2.0, for 90min and 60^{o}C, 3.85% @ pH 2.5, for 120 min and 70^{o}C. From the results it can be inferred that the pH could be the most important variable to increase the yield of pectin from *Lagenaria siceraria* as compared to time and temperature.

5.2 Quality analysis of pectin extracted from *Lagenaria siceraria:*

5.2.1 Moisture Content (%): The pectin sample obtained from the *Lagenaria siceraria* was analyzed for moisture content. The nature of pectin is hygroscopic i.e., it has the

tendency to absorb moisture in it from the air. The percentage of moisture in pectin sample also studied at three different pH @ pH=1.5 the mean value of moisture content observed was 14.53%, @ pH=2.0 the mean value of moisture content was 16.78% and at pH=2.5 the mean value of moisture content was 19.91% as shown in the Table 5.2.1. The mean values of moisture content for all the respective pH ranges 1.5 - 2.5 observed were quite high. After observing the result it can be told that the increasing pH may cause more water absorption (which seems to be easily vulnerable to microbial attack) and as such result in more yield. Because of this reason, it needs to be preserved in air tight container to avoid the moisture contact (Salam *et al*., 2012).

Table 5.2.1 Percentage moisture in pectin extracted from *Lagenaria siceraria* at pH (1.5, 2.0 and 2.5)

No. of replicates	pH		
	1.5	**2.0**	**2.5**
1.	13.40%	16.15%	18.15%
2.	13.65%	16.65%	19.16%
3.	15.16%	16.90%	20.65%
4.	15.90%	17.40%	21.66%
Mean	**14.53%**	**16.78%**	**19.91%**
S.D	**0.012007**	**0.005204**	**0.015567**

5.2.2 Ash Content (%): Ash content was determined for pectin samples which were obtained extracted *Lagenaria siceraria* at different pH conditions: 1.5, 2.0, and 2.5. Below are the readings of ash content of pectin at different pH. As shown in the table, at pH=0.5 obtained mean Ash content was 7.75%, at pH=2.0 the mean Ash content obtained was 10.83% and at pH=2.5 the mean Ash content obtained around 14.20% as shown in the table 5.2.2. After observing the table it can be told that the pH increase also because the ash percentage in pectin powder obtained the reason may be the methoxylation of the galactoruronic acids. The best range of ash percentage for gel formation should be 10% according to the resulted quoted by Salam *et al*. (2012) that it can form good quality of gel. Therefore, taking this parameter into consideration, the extracted pectin can be considered of good quality.

Table 5.2.2 Percentage ash in pectin extracted from *Lagenaria siceraria* at pH (1.5, 2.0, and 2.5)

No. of replicates	pH		
	1.5	**2.0**	**2.5**
1.	6.50%	9.95%	13.20%
2.	7.25%	10.70%	13.45%
3.	8.50%	10.95%	14.95%
4.	8.75%	11.70%	15.20%
Mean	**7.75%**	**10.83%**	**14.20%**
S.D	**0.010607**	**0.007171**	**0.010206**

5.2.3 Total solids (TS): The reading for the total solids has been represented in the table 5.2.3. The total solids percentage of polysaccharides i.e. pectin fibers was found more at low pH in comparison to high pH. That may be due to action of acid on the fiber and release of more of pure pectin for gelling. The highest mean value for the ash total solids percentage observed was 85.4725 percent at pH 1.5 followed by 83.225 percent at pH 2.0 and least shown at pH 2.5 of 79.995% ash percentage. The readings showed that the total solids content was enough for gelling but the quality varies depending upon other factors responsible for it.

Table 5.2.3 Percentage total solids (TS) in pectin extracted from *Lagenaria siceraria* at pH (1.5, 2.0, and 2.5)

No. of replicates	pH		
	1.5	**2.0**	**2.5**
1.	86.6 %	83.85 %	81.85 %
2.	86.35 %	83.35 %	80.84 %
3.	84.84 %	83.1 %	79.35 %
4.	84.1 %	82.6 %	78.34 %
Mean	**85.4725%**	**83.225%**	**79.9975 %**
S.D	**1.200705**	**0.520416**	**1.505886**

5.3 Statistical analysis: ANOVA (Analysis of Variance) was performed to check that is there any significant difference between the readings of the parameters of different quantitative properties. ANOVA helps to analyze the difference within and among the

group of means and their associated procedures. It helps to check the variation among and between the groups.

5.3.1 Effect of pH treatments on different physiochemical properties of extracted Pectin from different sources (*Lagenaria siceraria*, Apple and Standard Pectin):

The experiment was conducted to observe the effect of different treatments of pH on extracted pectin from *Lagenaria siceraria*. In this study, Pectin from *Lagenaria siceraria* was extracted and for the comparison of different characteristics of Pectin from *Lagenaria siceraria*. Standard pectin (Apple pectin from HiMedia) has been taken from the lab of DBT, TIET, Patiala. The Apple contains a good amount of pectin, so apple pectin was extracted through the same method as for pectin from *Lagenaria siceraria* for the comparison. The standard pectin was taken from the lab, DBT, TIET, Patiala. The statistical analysis was done in suitable software. Anova analysis showed that there was a significant effect of different treatments of pH on extracted Pectin from *Lagenaria siceraria*, apple pectin and standard pectin at 5% level of significance.

5.3.1.1 Equivalent weight: Experimental data of the Equivalent weight variation at different pH was recorded as given in the following table 5.3.1.1. ANOVA was performed on the data mentioned below in the table 5.3.1.1. The equivalent weight of pectin describes the degree of esterification which helps in confirming the property of pectin that is jelly-forming, high molecular weight pectin have better ability. The values of the table 5.3.1.1 were found to have higher molecular weight. It was also found that the equivalent weight increase with the increase in pH that may be because of more esterification of galactouronic sites. The means values observed for the pectin from *Lagenaria siceraria* was 9.7, apple pectin was 2.3 and the standard pectin was obtained with mean value of 0.52. So, the pectin from *Lagenaria siceraria* can be termed as good gelling agent because high degree of esterification as compared to apple and standard pectin.

Table 5.3.1.1 Equivalent weight of all the replicates of all the Pectin samples (*Lagenaria siceraria*, Apple and Standard Pectin)

No. of replicates	Equivalent weight of *Lagenaria siceraria* Pectin	Equivalent weight of Apple Pectin	Equivalent weight of Standard Pectin
1	2.5	4	0.19
2	3.1	0.6	0.21
3	4.3	0.9	0.24
4	6.2	1.3	0.28
5	6.9	1.4	0.31
6	8.8	1.7	0.37
7	9.3	1.9	0.43
8	10.1	2.4	0.61
9	14.4	2.9	0.69
10	16.8	3.2	0.92
11	17.1	3.6	0.99
12	17.7	4.1	1.02
AVG	**9.766666667**	**2.333333**	**0.521667**
SD	**5.534905**	**1.212311**	**0.313857**

5.3.1.1.1 Statistical analysis of equivalent weight of Pectin from different sources (*Lagenaria siceraria*, Apple and Standard Pectin): The data was collected from the software which is mentioned in the following tables showing degree of freedom, mean square and critical difference at 5%. The resulted values were found significant. Hence the pH has a positive significant effect on the equivalent weight of pectin from all the three samples from *Lagenaria siceraria*, apple and standard pectin. Pectin from all sources found to be lower equivalent weight or so.

Table 5.3.1.1.1 (a) Effect of pH (1.5, 2.0, and 2.5) on equivalent weight of *Lagenaria siceraria* pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	6.5037230	.398163E-01
TREATMENTS	3	.39809160	.459759E-01
INTERACTIONS	6	.86817420E-01	.796326E-01
ERROR	24	22328690E-02	

Table 5.3.1.1.1 (b) Effect of pH (1.5, 2.0, and 2.5) on equivalent weight of Apple pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	3.0497160	.131307
TREATMENTS	3	.58542320	.151620
INTERACTIONS	6	.89985470	.262613
ERROR	24	.24283670E-01	

Table 5.3.1.1.1 (c) Effect of pH (1.5, 2.0, and 2.5) on equivalent weight of Standard pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	5.8036320	.597561E-01
TREATMENTS	3	.39634390	.690004E-01
INTERACTIONS	6	.24906480E-01	
ERROR	24	.50292890E-02	

5.3.1.2 Methoxy content: Experimental data of the methoxy content of pectin from different sources at different pH recorded as given in the following Table 5.3.1.2. The methoxyl content is an important factor in controlling the setting time of pectin. The table showed that the *Lagenaria siceraria* pectin has a mean value of 0.895% that is quite lower than the apple pectin mean value i.e. 1.445833% and the highest value found was of apple pectin i.e. 1.64175%. The methoxyl content observed was also found to be complementary to the Equivalent weight results. The data revealed that the methoxylation is there but at very low concentration which may not render a good gel by *Lagenaria siceraria* pectin.

Table 5.3.1.2 Methoxy content of all the replicates of all the Pectin samples (*Lagenaria siceraria*, Apple and Standard Pectin)

No. of replicates	Methoxy content of *Lagenaria siceraria* Pectin	Methoxy content of Apple Pectin	Methoxy content of Standard Pectin
1	0.194	0.72	1.233
2	0.219	0.97	1.267
3	0.339	1.06	1.337
4	0.589	1.11	1.358
5	0.594	1.19	1.439
6	0.744	1.24	1.488
7	0.992	1.35	1.654
8	1.042	1.69	1.879
9	1.292	1.72	1.992
10	1.387	1.91	2.008
11	1.563	2.01	2.017
12	1.785	2.38	2.029
AVG	0.895	1.445833	1.64175
SD	0.533732	0.492959	0.323289

5.3.1.2.1 Statistical analysis of methoxy content of Pectin from different sources (Lagenaria siceraria, Apple and Standard Pectin): ANOVA was performed on the above data given in Table 5.3.1.2. The software has processed the above data and gives the readings which are mentioned in the Table 5.3.1.2 (a), (b) & (c). The data revealed that the pH had a significant effect on the methoxy content of the *Lagenaria siceraria* pectin.

Table 5.3.1.2.1 (a) Effect of pH (1.5, 2.0, and 2.5) on methoxy content of *Lagenaria siceraria* pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.87786870	.190033E-02
TREATMENTS	3	.64941410E-01	.219431E-02
INTERACTIONS	6	.71207680E-02	.380066E-02
ERROR	24	.50862630E-05	

Table 5.3.1.2.1 (b) Effect of pH (1.5, 2.0, and 2.5) on methoxy content of Apple pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.40478520	.891333E-02
TREATMENTS	3	.49825030E-01	.102922E-01
INTERACTIONS	6	.48116050E-02	.178267E-01
ERROR	24	.11189780E-03	

Table 5.3.1.2.1 (c) Effect of pH (1.5, 2.0, and 2.5) on methoxy content of Standard pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.76126100	.163748E-01
TREATMENTS	3	.14516190	.189080E-01
INTERACTIONS	6	.27781170E-01	.327497E-01
ERROR	24	.37765500E-03	

5.3.1.3 Total anhydrouronic acid content: Experiment data of the Total anhydrouronic acid content of Pectin from different sources at different pH recorded as given in the following Table 5.3.1.3. The Total anhydrouronic acid content was also found lesser than other apple and standard pectin means the gelling quality of the *Lagenaria siceraria* pectin is lesser as compared to apple and standard pectin.

Table 5.3.1.3 Total anhydrouronic acid content of all the replicates of all the Pectin samples (*Lagenaria siceraria*, Apple and Standard Pectin)

No. of replicates	Anhydrouronic acid content of *Lagenaria siceraria* Pectin	Anhydrouronic acid content of Apple Pectin	Anhydrouronic acid content of Standard Pectin
1	744	1240	1156
2	886	1267	1189
3	992	1325	1231
4	1006	1390	1265
5	1123	1452	1290
6	1198	1489	1311

7	1232	1543	1387
8	1302	1602	1423
9	1436	1671	1488
10	1533	1705	1596
11	1576	1789	1664
12	1642	1831	1699
AVG	1222.5	1525.333	1391.583
SD	286.3632	199.0748	184.8756

5.3.1.3.1 Statistical analysis of Anhydrouronic acid content of pectin from different sources (*Lagenaria siceraria*, Apple and Standard Pectin): ANOVA was performed on the above data given in Table 5.3.1.3. The software has processed the above data and gives the readings which are mentioned in the Table 5.3.1.3 (a), (b) & (c). The data revealed that the pH had a significant effect on the anhydrouronic acid content of the *Lagenaria siceraria* pectin in comparison to the apple and standard pectin.

Table 5.3.1.3.1 (a) Effect of pH (1.5, 2.0, and 2.5) on total anhydrouronic acid content of *Lagenaria siceraria* pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.87786870	.190033E-02
TREATMENTS	3	.64941410E-01	.219431E-02
INTERACTIONS	6	.71207680E-02	.380066E-02
ERROR	24	.50862630E-05	

Table 5.3.1.3.1 (b) Effect of pH (1.5, 2.0, and 2.5) on total anhydrouronic acid content of Apple pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.25689700	.190033E-02
TREATMENTS	3	.18717450E-01	.219431E-02
INTERACTIONS	6	.81380210E-04	.380066E-02
ERROR	24	.50862630E-05	

Table 5.3.1.3 (c) Effect of pH (1.5, 2.0, and 2.5) on total anhydrouronic acid content of Standard pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.28765870	.336471E-01
TREATMENTS	3	.12858070E-01	.388523E-01
INTERACTIONS	6	.44759120E-03	.672942E-01
ERROR	24	.15945430E-02	

5.3.1.4 Degree of Esterification: Experiment data of the Degree of Esterification of Pectin from different sources at different pH recorded is given in the following Table 5.3.1.4

Table 5.3.1.4 Degree of Esterification of all the replicates of all the Pectin samples (*Lagenaria siceraria*, Apple and Standard Pectin)

No. of replicates	Degree of esterification of *Lagenaria siceraria* Pectin	Degree of esterification of Apple Pectin	Degree of esterification of Standard Pectin
1	15.14	3.72	6.05
2	15.65	3.99	7.39
3	16.02	4.02	7.84
4	16.21	4.53	8.04
5	17.14	4.97	8.59
6	17.70	5.25	8.80
7	18.23	5.67	9.24
8	18.79	6.02	9.68
9	19.20	6.49	9.97
10	20.11	6.91	10.02
11	20.46	7.26	10.35
12	20.78	7.89	10.67
AVG	17.95	5.56	8.88
S.D	1.95	1.38	1.37

5.3.1.4.1 Statistical analysis of Degree of esterification of Pectin from *Lagenaria siceraria*, Apple and Standard pectin: The values in the table as mentioned below showed that the degree of esterification in the *Lagenaria siceraria* obtained pectin

doesn't have much effect of pH and other conditions. Hence the result can be termed as insignificant.

Table 5.3.1.4.1 (a) Effect of pH (1.5, 2.0, and 2.5) on Degree of esterification of *Lagenaria siceraria* pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.18148800	.201560E-02
TREATMENTS	3	10447180E-01	.232742E-02
INTERACTIONS	6	.13224280E-03	.403120E-02
ERROR	24	.57220460E-05	

Table 5.3.1.4.1 (b) Effect of pH (1.5, 2.0, and 2.5) on Degree of esterification of Apple pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	.95126720	.304201E-02
TREATMENTS	3	.59644060E-01	.351261E-02
INTERACTIONS	6	.80998740E-03	.608402E-02
ERROR	24	.13033550E-04	

Table 5.3.1.4.1 (c) Effect of pH (1.5, 2.0, and 2.5) on Degree of esterification of Standard pectin

Variables	D.F	M.S	CD (5%)
ENVIRONMENTS	2	1.8231350	NS
TREATMENTS	3	.89467880	NS
INTERACTIONS	6	.75769300	1.29494
ERROR	24	.59045010	

5.4 FTIR analysis:

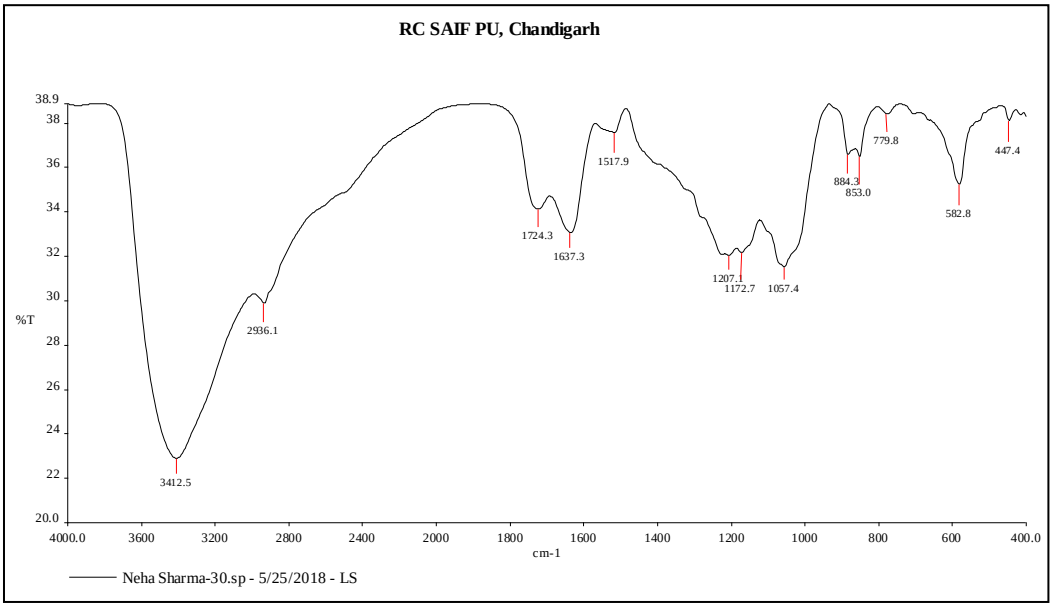

Fig.5.4 (a) FTIR Graph of *Lagenaria Siceraria* pectin

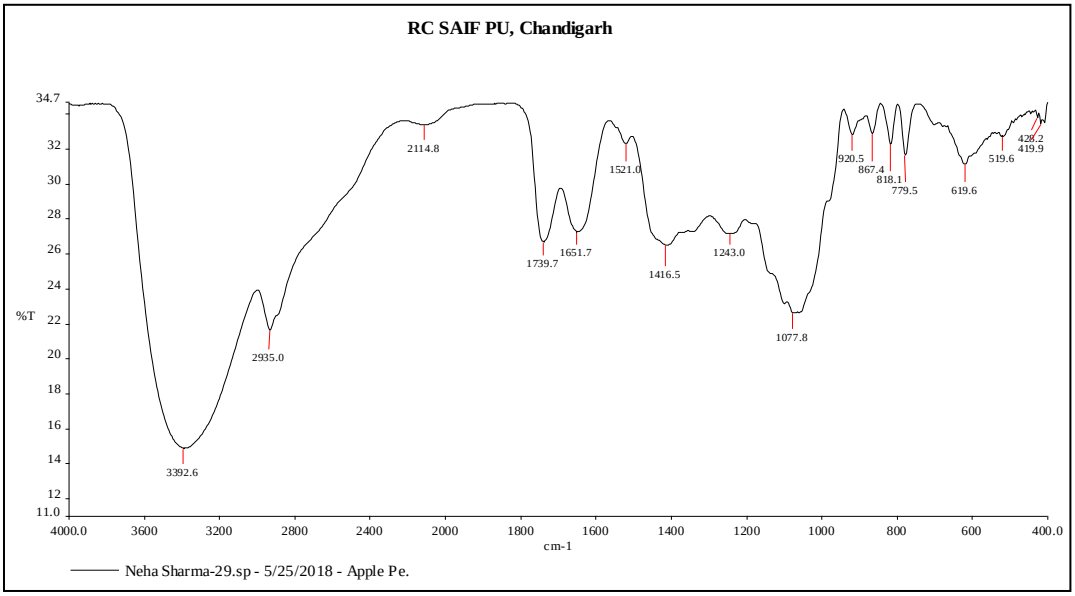

Fig.5.4 (b) FTIR Graph of Apple pectin

Confirming the characteristics of pectin extracted from *Lagenaria siceraria* and to evaluate the Degree of Esterification (DE)/presence of functional groups in the both apple pectin and *Lagenaria siceraria* samples they were examined by Fourier Transform Infrared Spectroscopy (FTIR). The figure 5.2 and 5.3 graphs itself revealed that the pectin spectra from apple and *Lagenaria siceraria* exhibited similarities in its absorption

pattern. The absorption area for broader, stronger range between 3600 and 2500 cm-1 refers to all over region of molecular hydrogen bonds absorption because of O±H stretching. The O±H extended vibrations appear in between a wide extent of frequencies that shows many attributes of a component involving hydroxyl groups extended bands which happens in sample are in the vapor phase and bonded O±H bands of carboxylic acid (Silverstein *et al.*, 1991). Taking samples of pectin into consideration, absorption in the O±H region is because of the molecular hydrogen bonding of the galacturonic acid polymer. Excellent bands occurring at the longer end of the O±H region shows overtones and combination of tone. In fig 5.3 apple pectin FTIR spectrum with the wavelength range of 920.5 and 1243 cm-1 can be taken as the 'finger print' area for carbohydrates as it gives the recognition of main chemical elements present in polysaccharides (Cernà *et al*., 2003). Resemblance of the pectin extracted from *Lagenaria siceraria* with the extracted apple pectin spectra in the "fingerprint" region shows that the sample is effectively pectin**.**

The Band spectra ranged 2950 cm^{-1} (3000±2800 cm-1) may refer to C±H. In pectin samples, the C±H stretching and bending vibrations were seen usually, as a band super-imposed on the wider O±H band that is between 2500 to 3600 cm^{-1} as can be seen in fig 5.2 and 5.3. Esterified pectin has an O±CH_3 extended band that was supposed to be in between 2950 and 2750 cm^{-1} due to methyl esters of galacturonic acid as can be seen in fit 5.3 of apple pectin. But because of the large O±H stretching response appearing in a broad region (3600± 2500 cm^{-1}), the O±CH_3 activity is covered and it is a non-dependable measure of methoxylation. Stronger bands appearing in the range of 1760±1745 cm-1, and 1640 and 1620 cm^{-1} show the ester carbonyl (C.O) groups and carboxylate ion stretching band (COO±), as describe below in fig 5.2 and 5.3. It was seen that the ester carbonyl groups rises in their intensity and band area as the DE increased, while the intensity of the carboxylate extended band reduced in fig 5.2 and 5.3. For quantitative pectin analysis, the O±CH_3 stretching bands are not useful, the bands showing ester carbonyl (1760± 1745 cm^{-1}) and free carboxylate groups (1640±1620cm^{-1}) are sufficient for the identification and quantitation of pectin samples. Carboxylate groups have two bands, an asymmetrical stretching band close to 1650±1550 cm^{-1}, and a poor symmetric stretching band close to 1400 cm^{-1} as in case of apple pectin fig 5.2. In

pectin sample fig 5.3, the poor symmetric COO± stretching is followed by moderately intense absorption patterns between 1300 and 800 cm^{-1}, altogether referred to as the 'finger print" area that is quite different to a compound. These bands are usually di-cult to interpret. Other bands of lesser significance in pectin samples are C±H bending, occurring at 1380 cm^{-1}, and C±O stretching occurring at 1300±1000 cm^{-1}.

DE of Pectin can be evaluated by using the peak area relation of the free carboxyl groups (1650 cm^{-1}) and esterified groups (1750 cm^{-1}) (Gnanasambandan *et al*., 2000) It was calculated by using the peak areas values of these bands using the following expression:

{DE = area of esterified carboxyl groups/(area of esterified carboxyl groups + area of non-esterified carboxyl groups) × 100}

Figure 5.3, represents the spectrum of FTIR of apple pectin in which the absorbance is elevated at 1739.1 cm-1 near to 1750 cm-1 than at 1651.7~1650cm-1, which is main feature of high methoxyl pectin (Gnanasambandan *et al*., 2000). Figure 5.2 represents the spectrum of FTIR of *Lagenaria siceraria* pectin presenting a lower absorbance than 1750 cm-1 i.e. 1724.3 than at 1637.3 again which is lower than 1650 cm-1, showing Low methoxy pectin. The Degree of esterification of *Lagenaria siceraria* pectin was estimated to be about 10%.

5.5 XRD analysis:

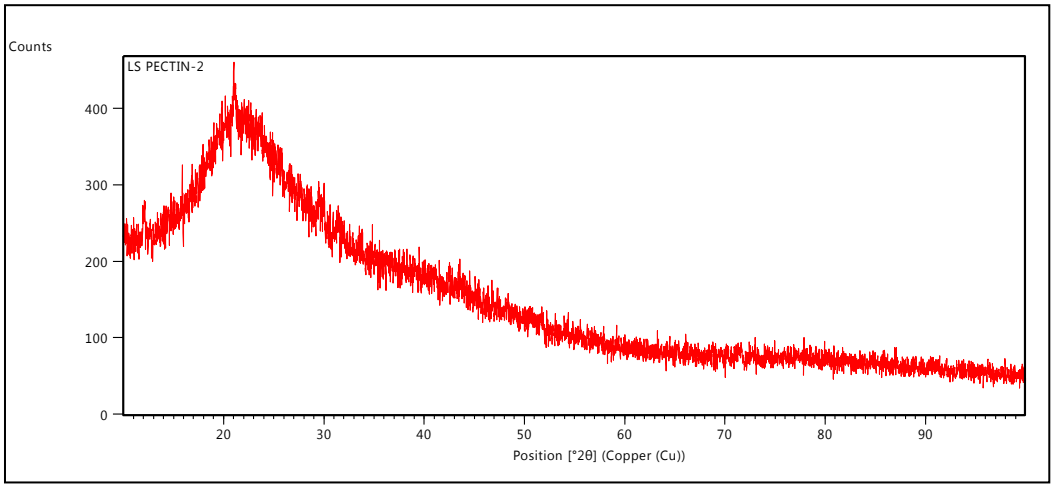

Fig.5.5 (a) XRD Graph of *Lagenaria siceraria* Pectin

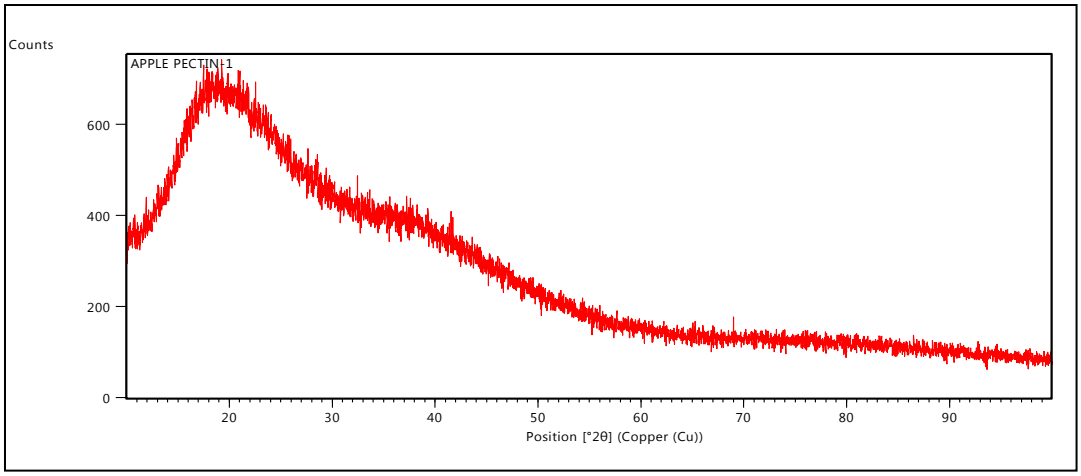

Fig.5.5 (b) XRD Graph of Apple Pectin

X-ray diffraction profiles of micro-particles were taken from the methodology reported by Ratnayake and Jackson, 2008). Investigation of these gives a better idea on the whole structure, such as crystalline or amorphous natures, of the particles. Pectin samples were examined under the mentioned data: omega = 4°, detector swing angle = 18°, sample to detector distance = 20cm and exposure time = 180s. Pattern of X-ray diffraction of pectin in fig 5.4 and 5.5, however, revealed a major peak at approximately in between 19-22° 2θ. And the same has been described by Lutz *et al*., 2009 of pectin XRD where patterns of X-ray diffraction of pectin sample attained a major peak at 21° 2θ. (Lutz *et al*., 2009) reported similar results and XRD profiles for apple pectin. So, it can be told that the quality of pectin obtained from *Lagenaria siceraria* in comparison to apple pectin was of good quality.

CHAPTER-VI

CONCLUSION

The method for pectin extraction from *Lagenaria siceraria* was optimized using acid extraction method as used by Srivastava P *et al*., 2011. To optimize the pH 0.1 M of H_2SO_4 was used to 1.5, 2.0 and 2.5 values with variation in time of incubation for 1 hr, 2 hrs and 3 hrs simultaneously the temperature was also optimized at 50^oC, 60^oC and 70^oC respectively. A significant effect was observed on the yield of the pectin percentage from *Lagenaria siceraria* to the control sample (apple pectin) with a gradual increase in pH-1.5, 2.0 and 2.5. The ascending pH variation was observed with a positive significant effect on equivalent weight (*Lagenaria siceraria* pectin-9.7, Apple pectin-2.3 & Standard pectin-0.5), methoxyl content (*Lagenaria siceraria* pectin-90.8, Apple pectin-1.4 & Standard pectin-1.6), total anhydrouronic acid content (*Lagenaria siceraria* pectin-1222.5, Apple pectin-1525.3 & Standard pectin-1391.6) and degree of esterification (*Lagenaria siceraria* pectin-17.9, Apple pectin-5.6 & Standard pectin-8.9). The pectin obtained from *Lagenaria siceraria* was found to be less in percentage to whole piece but in comparison to the quality for gel making quite promising nearby to that of apple pectin as observed through the FTIR and XRD results. It would not be wrong to say that the *Lagenaria siceraria* can be a cheaper source for pectin extraction but the quantity is less however overall quality can be exploited to provide best results.

ANNEXURE-I (LIST OF FIGURES)

ANNEXURE-II (LIST OF TABLES)

ANNEXURE-III (LIST OF ABBREVIATIONS)

HG	Homo-galacturonan
XGA	Xylo-galaturonan
RG	Rhamno-galacturonan
DE	Degree of Esterification
HM Pectin	High-Methoxy Pectin
LM Pectin	Low-Methoxy Pectin
Kcal	Kilo Calories
DM	Degree of methoxylation
TS	Total Solids
AUA	Anhydrouronic acid
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray diffraction
FAO	Food and Agriculture Organization
DF	Degree of Freedom
MS	Mean Square
CD	Critical Difference

REFERENCES

1. Aina V O, Barau M M, Mamman O A, Zakari A, Haruna H, Umar M H & Abba Y B. (2012). Extraction and characterization of pectin from peels of lemon (*Citrus limon*), grape fruit (*Citrus paradisi*) and sweet orange (*Citrus sinensis*). British Journal of Pharmacology and Toxicology. 3(6): 259-262.
2. Albuquerque P, Coelho L C, Teixeira J A & Carneiro-da-Cunha M G. (2016). Approaches in biotechnological applications of natural polymers. AIMS Molecular Science. 3(3): 386-425.
3. AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.
4. Aydin Z & Akbugˇa J. (1996). Preparation and evaluation of pectin beads. International Journal of Pharmaceutics. 137(1): 133-136.
5. Bruso J. (2017). "List of Foods Containing Pectin." Healthfully. https://healthfully.com/list-foods-containing-pectin-5903391.html.
6. Calabrese N, Venere D & Linsalata V. (1997). Technological and qualitative aspects of calabash gourd, *lagenaria siceraria* (molina) standley, for processing. In I International Symposium on Cucurbits. 492:179-186.
7. Cañasab NA, Wolf S, Wagner N and Friedrich A. (2013). In-situ X-ray diffraction studies of lithium–sulfur batteries. Journal of Power Sources. 226 (15): 313-319.
8. Černá M, Barros A S, Nunes A, Rocha S M, Delgadillo I, Čopíková J & Coimbra M A. (2003). Use of FT-IR spectroscopy as a tool for the analysis of polysaccharide food additives. Carbohydrate Polymers. 51(4): 383-389.
9. Chan SY, Choo WS, Young DJ, Loh XJ. (2017). Pectin as a rheology modifier: Origin, structure, commercial production and rheology. Carbohydrate Polymers. 161:118-39.
10. Christensen SH. (2014). Engineering in Context: Structural, Cultural, Social and Epistemological Tensions in Engineering Education.
11. Christiaens S, Van Buggenhout S, Houben K, Jamsazzadeh Kermani Z, Moelants K R, Ngouemazong ED, & Hendrickx M E. (2016). Process–structure–function

relations of pectin in food. Critical Reviews in Food Science and Nutrition, Food Science. 56(6):1021-1042.

12. Colin D. May, H. P. Bulmer. (2002). Industrial Pectins: Sources, Production and Applications.
13. Dixon DW. (2008). Characterization of Commercial Pectin Preparations Spectroscopic and Chromatographic Techniques. Electronic Theses and Dissertations.
14. Fellah A, Anjukandi P, Waterland M R & Williams M A. (2009). Determining the degree of methylesterification of pectin by ATR/FT-IR: Methodology optimisation and comparison with theoretical calculations. Carbohydrate Polymers. 78(4): 847-853.
15. Filippov MP, Shkolenko GA and Kohn R. (1978). Determination of the esterification degree of the pectin of different origin and composition by the method of infrared spectroscopy. Chemical Papers 32(2): 218–222.
16. Fishman M L & Chau H K. (2000). U.S. Patent No. 6,143,337. Washington, DC: U.S. Patent and Trademark Office.
17. Flourie B, Vidon N, Florent C H & Bernier J J. (1984). Effect of pectin on jejunal glucose absorption and unstirred layer thickness in normal man. Gut. 25(9): 936-941.
18. Fourie P C. (1996). Fruit and human nutrition. In Fruit processing. Springer, Boston, MA. 20-39.
19. Girma E & Worku T. (2016). Extraction and characterization of pectin from selected fruit peel waste. International Journal of Scientific and Research Publications. 6(2): 447-454.
20. Girma E & Worku T. (2016). Extraction and characterization of pectin from selected fruit peel waste. International Journal of Scientific and Research Publications. 6(2): 447-454.
21. Gnanasambandam R & Proctor A. (2000). Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. Food Chemistry. 68(3): 327-332.
22. Ismail N S M, Ramli N, Hani N M & Meon Z. (2012). Extraction and characterization of pectin from dragon fruit (*Hylocereus polyrhizus*) using various extraction conditions. Sains Malaysiana. 41(1): 41-45.

23. Jain RK, Ghankrokta SS and Agrawal JD, (1984). Isolation and characterization of pectin from apple pomace. Indian Food Packer. 38: 65–70.
24. Kaur G. (2014). Development of mixed Vegetable health Beverages (Doctoral dissertation, Punjab Agricultural University, Ludhiana).
25. Kertesz ZI (1951). The pectic substances. New York: Interscience. 438.
26. Khule NR, Mahale NB, Shelar DS, Rokade MM, Chaudhari SR, (2012). Extraction of pectin from citrus fruit peel and use as natural binder in paracetamol tablet. Der Pharmacia Lettre. 4(2): 558–564.
27. Kirtil E, Oztop H.M, Sirijariyawat A, Ngamchuachit P' Barrett D.M, McCarthy M.J, (2004). Effect of pectin methyl esterase (PME) and $CaCl_2$ infusion on the cell integrity of fresh-cut and frozen-thawed mangoes: An NMR relaxometry study. Food Research International. 66: 409-416.
28. Kohn R, (1982). Binding of toxic cations to pectin, its oligomeric fragments and plant tissues, Carbohydrate Polymer. 2:273-275.
29. Kyomugasho C, Christiaens S, Shpigelman A, Van Loey A M & Hendrickx M E. (2015). FT-IR spectroscopy, a reliable method for routine analysis of the degree of methylesterification of pectin in different fruit-and vegetable-based matrices. Food Chemistry. 176: 82-90.
30. Levigne S V, Ralet M C J, Quéméner B C, Pollet B N L, Lapierre C & Thibault J F J (2004). Isolation from sugar beet cell walls of arabinan oligosaccharides esterified by two ferulic acid monomers. Plant Physiology. 134(3):1173-1180.
31. Liew S Q, Chin N L & Yusof Y A. (2014). Extraction and characterization of pectin from passion fruit peels. Agriculture and Agricultural Science Procedia. 2: 231-236.
32. Lima M S, Paiva E P, Andrade S A C & Paixão J A. (2010). Fruit pectins–A suitable tool for screening gelling properties using infrared spectroscopy. Food Hydrocolloids. 24(1): 1-7.
33. Liu L, Fishman M L, Kost J & Hicks K B. (2003). Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials. 24(19): 3333-3343.
34. Liu P & Krishnan T R. (1999). Alginate- pectin- poly- L- lysine particulate as a potential controlled release formulation. Journal of Pharmacy and Pharmacology. 51(2): 141-149.

35. Lutz R, Aserin A, Wicker L & Garti N. (2009). Structure and physical properties of pectins with block-wise distribution of carboxylic acid groups. Food Hydrocolloids. 23(3): 786-794.
36. Manabe M and Tirutani T. (1966). Extraction and determination of pectic substances, Chemical Abstracts. 65: 10786.
37. May C D. (1990). Industrial pectins: sources, production and applications. Carbohydrate polymers. 12(1): 79-99.
38. Miceli-Garcia L G. (2014). Pectin from Apple Pomace: Extraction, Characterization, and Utilization in Encapsulating Alpha-Tocopherol Acetate.
39. Miettinen T A & Tarpila S. (1977). Effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. Clinica Chimica Acta. 79(2): 471-477.
40. Modgil M, Modgil R & Kumar R. (2004). Carbohydrate and mineral content of chyote (*Sechium edule*) and bottle gourd (*Lagenaria siceraria*). Journal of Human Ecology.15(2): 157-159.
41. Monsoor M A, Kalapathy U & Proctor A. (2001). Determination of polygalacturonic acid content in pectin extracts by diffuse reflectance Fourier transform infrared spectroscopy. Food Chemistry. 74(2): 233-238.
42. Munarin F, Tanzi M C & Petrini P. (2012). Advances in biomedical applications of pectin gels. International journal of biological macromolecules. 51(4): 681-689.
43. Novosel'skaya I L, Voropaeva N L, Semenova L N & Rashidova S S. (2000). Trends in the science and applications of pectins. Chemistry of Natural Compounds. 36(1): 1-10.
44. Okimasu S. (1956). A new method for the quantitative determination of pectin in plant materials by colloid titration. Bulletin of the Agricultural Chemical Society of Japan. 20(1): 29-35.
45. Pandharipande S & Makode H. (2012). Separation of oil and pectin from orange peel and study of effect of pH of extracting medium on the yield of pectin. Journal of Engineering Research and Studies. 3(2): 06-09.

46. Pawar H A, Kamat S R & Choudhary P D. (2015). An overview of natural polysaccharides as biological macromolecules: their chemical modifications and pharmaceutical applications. Biology and Medicine. 7(1): 1.
47. Pilnik W. and Voragen A. (1992). Gelling agents (pectins) from plants for the food industry, Mueller-Harvey, I., McAllan, A. B., & Morrison, I. M. (1992). Advances in Plant Cell Biochemistry and Biotechnology.
48. Prasanna V, Prabha T N & Tharanathan R N. (2007). Fruit ripening phenomena: An overview. Critical Reviews in Food Science and Nutrition. 47(1): 1-19.
49. Pruthi J S, Parekh C M and Lal G. (1961). An integrated process for the recovery of essential oil and pectin from Mandarin orange waste. Food Science. 10 (11): 372–378.
50. Rahman A H. (2003). Bottle Gourd (Lagenaria siceraria)-a vegetable for good health. Natural Product Radiance. 2(5): 249-250.
51. Ranganna S, (1995). Hand book of analysis and quality control for fruits and vegetable products (2nd Ed.). New Delhi: McGraw Hill publishing Co. Ltd. pp: 33-43.
52. Ratnayake W S & Jackson D S. (2007). A new insight into the gelatinization process of native starches. Carbohydrate Polymers. 67(4): 511-529.
53. Ratnayake W S & Jackson D S. (2008). Thermal behavior of resistant starches RS 2, RS 3, and RS 4. Journal of food science. 73(5): C356-C366.
54. Ratnayake W S, Otani C & Jackson D S. (2009). DSC enthalpic transitions during starch gelatinisation in excess water, dilute sodium chloride and dilute sucrose solutions. Journal of the Science of Food and Agriculture. 89(12): 2156-2164.
55. Ratnayake WS, Jackson DS. (2009). Starch gelatinization. In: Taylor SL, Editor. Advances in food and nutrition research. Amsterdam, the Netherlands: Academic Press. Pp: 221–68.
56. Roopan S M, Rajeswari V D, Kalpana V N & Elango G. (2016). Biotechnology and pharmacological evaluation of Indian vegetable crop *Lagenaria siceraria*: An overview. Applied Microbiology and Biotechnology. 100 (3): 1153-1162.
57. Rouse A.H and Crandal P G. (1976). Nitric Acid extraction of pectin from citrus peel. Proceedings of Florida State Horticulture Society. 89:166–168.

58. Sakai T & Okushima M. (1980). Microbial production of pectin from citrus peel. Applied and Environmental Microbiology. 39(4): 908-912.
59. Salma M A, Jahan N, Islam M A & Hoque M M. (2012). Extraction of Pectin from lemon peel: Technology development. Journal of Chemical Engineering. 27(2): 25-30.
60. Salma M A, Jahan N, Islam M A & Hoque M M. (2012). Extraction of Pectin from lemon peel: Technology development. Journal of Chemical Engineering. 27(2): 25-30.
61. Sandarani MDJC. (2017). A Review: Different Extraction Techniques of Pectin. Journal of Pharmacognosy & Natural Products. 3:3 DOI: 10.4172/2472-0992.1000143.
62. Sangam, P. (n.d) Essay on Cell Wall (For School and College Students). http://www.indiaessays.com/essays/india/biology/cell-wall/essay-on-cell-wall-for-school-and-college-students-cell-biology/14539
63. Shahnawaz M & Shiekh S A. (2011). Analysis of viscosity of jamun fruit juice, squash and jam at different compositions to ensure the suitability of processing applications. International Journal of Plant Physiology and Biochemistry. 3(5): 89-94.
64. Sharma B R, Naresh L, Dhuldhoya N C, Merchant S U & Merchant U C. (2006). An overview on pectins. Times Food Processing Journal. 23(2): 44-51.
65. Silverstein, R. M., & Bassler, G. C. (1962). Spectrometric identification of organic compounds. Journal of Chemical Education, 39(11): 546.
66. Slany J, Bronisa D, Chorvatova B & Mandak M. (1981). Evaluation of tablets with pectin as a binding agent. Pharmaceutically Obzor. 50: 491-498.
67. Slany J. (1981). Study of functional action of citrus pectins in tablets. Czech and Slovak Pharmacy Magazine. 30: 195-200.
68. Sriamornsak P, Prakongpan S, Puttipipatkhachorn S & Kennedy R A. (1997). Development of sustained release theophylline pellets coated with calcium pectinate. Journal of Controlled Release. 47(3): 221-232.
69. Sriamornsak P, Puttipipatkhachorn S & Prakongpan S. (1997). Calcium pectinate gel coated pellets as an alternative carrier to calcium pectinate beads. International Journal of Pharmaceutics. 156(2):189-194.

70. Sriamornsak P. (2003). Chemistry of pectin and its pharmaceutical uses: A review. Silpakorn University International Journal. 3(1-2): 206-228.
71. Srirangarajan A N & Shrikhande A J. (1977). Characterization of mango peel pectin. Journal of Food Science. 42(1): 279-280.
72. Srivastava P & Malviya R. (2011). Sources of pectin, extraction and its applications in pharmaceutical industry: An overview. Indian Journal of Natural Products and Resources. Vol. 2(1): 10-18.
73. Stephen, A. (Ed.), Phillips, G. (Ed.), Thompson, J., Skjak-Braek, G., Wurzburg, M., Rao, M., Bell, D., Brummer, Y., Gidley, M., Chums, S., Blanchard, P., Katz, F., Coffey, D., Henderson, A., Stanley, N., Moe, S., Chesson, A., Williams, P., Morris, V., Cui, S., Varum, K., van Aken, G., Tolstoguzov, V., Reid, J., Piculell, L., Draget, K., Franck, A., Lopes da Silva, J., Smidsrod, O., Zobel, H., Nishinari, K. (2006). Food Polysaccharides and Their Applications. Boca Raton: CRC Press.
74. Sungthongjeen S, Pitaksuteepong T, Somsiri A & Sriamornsak P. (1999). Studies on pectins as potential hydrogel matrices for controlled-release drug delivery. Drug Development and Industrial Pharmacy. 25(12): 1271-1276.
75. Thakur B R, Singh R K, Handa A K & Rao M A. (1997). Chemistry and uses of pectin: A review. Critical Reviews in Food Science & Nutrition. 37(1): 47-73.
76. Tiwari AK, Saha SN, Yadav VP, Upadhyay UK, Katiyar D, Mishra T. (2017). Extraction and Characterization of Pectin from Orange Peels. International Journal of Biotechnology and Biochemistry. Vol. 13(1): 39-47.
77. Tyagi V, Sharma P & Malviya R. (2015). Pectins and their role in food and pharmaceutical industry: A review. Journal of Chronotherapy and Drug Delivery. 6(3): 65-77.
78. Voragen, AGJ., Pilnik, W., Thibault, J-F., Axelos, MAV., Renard, CMGC. (1995) .Pectins. In Stephen AM (ed) Food Polysaccharides and their applications. Marcel Dekker, Inc, New York, pp 287–339.
79. Wong T W, Chan L W, Lee H Y & Heng P W S. (2002). Release characteristics of pectin microspheres prepared by an emulsification technique. Journal of Microencapsulation. 19(4): 511-522.

80. Xia H & Matharu A S. (2017). Unavoidable food supply chain waste: acid-free pectin extraction from mango peel via subcritical water. Faraday Discussions. 202: 31-42.
81. www.peta.org/food/gelatin-alternatives/
82. http://freshokartz.com/fruits-vegetables/vegetables/bottle-gourd.html
83. http://sansho.co.jp/en/find/polysaccharide/pectin/
84. http://www.cybercolloids.net
85. http://www.plantphysiol.org.
86. https://healthfully.com/list-foods-containing-pectin-5903391.html
87. https://www.businesswire.com/news/home/20170306005571/en/Global-Pectin-Market-Driven-High-Demand-Generated
88. https://www.sciencedirect.com/science/article/pii/S0963996914006528
89. https://www.slideshare.net/dramrhelal/stdf-pectin-presentation

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