Development Process Protocol for Extraction of Pectin from Different Fruit Peel

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THESIS

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2019

DECLARATION

I, hereby declare that this thesis entitled ***"Development Process Protocol for Extraction of Pectin from Different Fruit Peel"*** is a bonafide record of research work done by us during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **" Development Process Protocol for Extraction of Pectin from Different Fruit Peel "** is a record of research work done independently by Ms. Athira V B (2016-06-009), Ms. Greeshma P R (2016-06-013), Ms.Reshma M (2016-06-018), Ms. Silpa A S (2016-06-023) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Athira V B

Greeshma P R

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Dedicated to

Our Parents and

Teachers

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LIST OF SYMBOLS AND ABBREVATIONS

%	:	per cent
&	:	And
/	:	Per
=	:	equal to
±	:	plus or minus
≈	:	Approximate
3D	:	Three dimensional
AUA	:	Anhydrouronic acid content
D	:	dextro rotatory
d.b	:	dry basis
DE	:	degree of esterification
DM	:	degree of methylation
et al.	:	and others
etc.	:	Etcetera
Fig.	:	Figure
g	:	Gram
h	:	hour
HCL	:	hydro chloric acid
HG	:	Homogalacturonan
HM	:	high methoxyl
HMP	:	high methoxyl pectin
K.C.A.E.T	:	Kelappaji College of Agricultural Technology

KAU	**:**	**Kerala Agricultural University**
Kg	**:**	**kilo gram**
L	**:**	**Litter**
L	**:**	**Levo rotatory**
LM	**:**	**Low methoxyl**
MeO	**:**	**Methoxy group**
Min	**:**	**Minute**
ml	**:**	**milli litre**
MT	**:**	**million tonne**
NaOH	**:**	**sodium hydroxide**
No.	**:**	**Number**
^{o}B	**:**	**Brix**
^{o}C	**:**	**Degree Celsius**
PET	**:**	**poly ethylene terephthalate**
rpm	**:**	**revolution per minute**
s	**:**	**second**
t	**:**	**tonne**
TSS	**:**	**Total soluble solids**
USA	**:**	**United states of America**
w.b	**:**	**wet basis**
wt.	**:**	**weight**

INTRODUCTION

CHAPTER 1

INTRODUCTION

In India, there are lots of food industries. These industries produce different types of foods. There are some food industries, those use fruits for food preparation and processing of different kinds of fruit juice, jam, jelly, chocolates, bar and so on. These industries mainly used seasonally available fruits. On the other hand, large volume of fruit wastes is produced from these food industries. Fruit wastes are highly perishable and seasonal. People of our country especially industrial people cannot properly handle fruit wastes, that's why it's a problem to the processing industries (Apsara & Pushpalatha, 2003).

These wastes lead to serious pollution problem in the environment and it also represent a loss of valuable nutrients and biomass (Chacko & Estherlydia, 2014). Environmental pollution problem can be reduced by by-product recovery from fruit wastes. It also improves the overall economy of the processing unit of a food processing industry. Pectin is a valuable by-product which can be extracted from fruit wastes. Pectin is an important element which is found in the cell wall of the fruit. If food industries use the fruit peel for the extraction of pectin, then it will reduce the pollution problem.

The main things about pectin is it has a good gelling property, which is used in different food preparation and many more things (Apsara & Pushpalatha, 2003). Pectin is used in manufacture of jams, jellies, marmalades, preserves etc. It is also useful as a thickening agent for sauces, ketchups, flavored syrups and as a texture agent in fruit-flavored milk deserts. Besides, it has numerous applications in pharmaceutical preparations, pastes, cosmetics etc. But, the single largest use of pectin is in the manufacture of jellies. Most of the commercial pectin in the world is used to make jelly and similar products.

In our country, most of the jam or jelly production industries use commercial pectin. These industries brought pectin from other countries to produce good quality of product. Thus, it also increases the production cost. From our current understanding, we found this one as a gap in our food industry which need to be addresses. In our study, we extracted pectin from our low-cost fruit wastes and used that pectin to prepare jelly. The present study was therefore designed to estimate pectin from available fruit wastes such as Jackfruit peel,

Passion fruit peel and Pineapple peel. Commercially available pectin is mainly extracted from Apple, so for the comparison purpose we use pectin extracted from the Apple.

Pineapple (*Ananas comosus)* is one of the commercially important fruit crops of India. Total annual world production is estimated at 19412.91 thousand tons of fruits. India is the fifth largest producer of pineapple with an annual output of about 1415 thousand tons. It is abundantly grown in almost entire North East region, West Bengal, Kerala, Karnataka, Bihar, Goa and Maharashtra states. With the increase in production of processed fruit products, the amount of fruit wastes generated is increasing enormously. Rajibul *et al.*,(2014) reported that postharvest losses in pineapple are 40 percent due to poor handling and indiscriminate use of growth promoting and ripening agents.

Fig 1.1. *Ananas comosus*

Jackfruit (*Artocarpus heterophyllus Lam.*) trees belong to the family **"Moraceae".** It is locally known as **"Kathal"**. They grow abundantly in India, Bangladesh, and in many parts of Southeast Asia. It is considered as one the most significant evergreen trees in tropical areas and widely grown in Asia including India. Jackfruit is a good source of protein, starch, calcium, and thiamine (Swami *et al*., 2012).

Fig 1.2. *Artocarpus heterophyllus Lam*

The passion fruit (*Passiflora edulis*), family Passifloraceae, is a native of Brazil. In India passion fruit cultivation is confined to Kerala, Tamil Nadu, Karnataka, and North Eastern states with an area and production of 9.11 thousand ha and 45.82 thousand tons. It has many pharmacological properties. It is used primarily for fresh consumption and the production of juice. It has become increasingly popular all over the world and it is used in jams, jellies and juices.

Fig 1.3. *Passiflora edulis*

Apple (*Malus pumila*) is an important temperate fruit. Apples are mostly consumed fresh but a small part of the production is processed in to juices, jellies, canned slices and other items.

In India, Apple is primarily cultivated in Jammu & Kashmir; Himachal Pradesh; hills of Uttar Pradesh and Uttaranchal. It is also cultivated to a small extent in Arunachal Pradesh; Nagaland; Punjab and Sikkim. The estimated total production of apples in India was 2265000 tons with an area of production 305000 hectares (2017).

Fig 1.4. *Malus pumila*

1.2 OBJECTIVE

The main objectives of this project were to determine the feasibility of pectin extraction from fruit peel through optimization of pectin extraction methods. The methods chosen for optimization were acid extraction.

The specific objectives were to:

1. To use the fruit peels for the extraction of pectin in order to enhance the waste utilization as well as controlling the environmental pollution.
2. To investigate the acid extraction procedures to produce the highest obtainable yield from different fruit peels.
3. To compare the yield of pectin extracted from different fruit waste and to analyze its physico-chemical properties.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 PECTIN

All fruit peels contain pectin at their cell wall and some pectin is also found in fruit pulp. Pectin is an important factor during fruit ripening, and the amount of pectin varies in different types of fruits. Pectin is derived from heteropolysaccharides, which is extracted from the primary cell wall of higher plants. Pectin is a functional ingredient in the food industry because it has a good gelling ability and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products. Also, the pharmaceutical industries widely use pectin. It has been reported that, pectin lower the blood cholesterol levels and low density lipoprotein cholesterol fractions, which is beneficial for human health. It is also stated that, pectin may help decrease tumor cell formation (Bhat *et al*., 2014). Pectin can also be used in several ways like biodegradable water-soluble films, bulking agents, coating agents, chelators, emulsifiers and viscosity modifiers (Kanmani *et al*., 2014).

2.2 GENERAL PROPERTIES OF PECTIN

Pectin are soluble in pure water. Monovalent cation salts which are pectinic and pectic acids are usually soluble in water; di and trivalent cations salts are weakly soluble or insoluble. When dry powdered pectin are added to water it hydrates very rapidly, forming clumps. These clumps are of semidry packets of pectin contained in an envelope of highly hydrated outer coating. Clump formation can be prevented by dry mixing pectin powder with water-soluble carrier material (Whistler and BeMiller,1993).

The nature of dilute pectin solutions is Newtonian but at a moderate concentration, they exhibit the non-Newtonian, pseudo plastic behavior. Viscosity, solubility, and gelation are generally related with these. For example, factors that increase gel strength will increase the tendency of gel formation, decrease solubility, and increase viscosity. These properties are the function of pectin structure, which is that of a linear poly anion. Such as, in solution some monovalent cation salts of pectin are highly ionized, and the distribution of ionic charges in the molecule tends to keep it in an extended form by coulombic repulsion (Paoletti, 1986).

This same coulombic repulsion act between the carboxylate anions to prevent aggregation of the polymer chains. In addition, each polysaccharide chain, especially each carboxylate group, will be highly hydrated. Monovalent salts of pectin solution exhibit stable viscosity because each polymer chain is hydrated, extended, and independent. If the pH is lowered then ionization of the carboxylate group is decreased, and this results in a reduction in hydration of the carboxylic acid groups. As a result of reduced ionization, the polysaccharide molecules no longer repel each other, that's why they can associate and form a gel. Apparent pK values vary with the DE of the pectin (Plashchina *et al*., 1978); a 65% DE pectin has an apparent pK of 3.55, while a 0% DE pectic acid has an apparent pK of 4.10.

The main use of pectin is based on its ability to form gels. Pectin forms gel with sugar and acid. Gel is formed by hydrogen bonding between free carboxyl groups on the pectin molecules and between the hydroxyl groups of neighboring molecules. In a neutral or only slightly acid dispersion of pectin molecules, most of the un-esterified carboxyl groups are present as partially ionized salts. When acid is added then the carboxyl ions are converted to mostly unionized carboxylic acid groups. This reduce the number of negative charges not only lowers the attraction between pectin and water molecules, but also lowers the repulsion between pectin molecules. For competing water sugar further decreases hydration of the pectin. The degree of esterification is also affected by the rate of gel formation. Rapid setting of gel is caused by the higher DE. Rapid-set pectin (i.e. pectin with a DE of above 72%) also gel at lower soluble solids and higher levels than slow-set pectin's (i.e. pectin with a DE of 58- 65%) (Raj *et al*., 2012).

Pectin has also applications in the pharmaceutical industry. Pectin reduces cholesterol levels in blood (Sriamornsak, 2001). Consumption of at least 6 g/day of pectin is necessary to have a significant effect in cholesterol reduction. Amounts less than 6 g/day of pectin are not effective for cholesterol reduction (Ginter *et al*.,1979).

2.2.1 Backbone structure

Pectins are a family of complex polysaccharides which contain 1,4-linked α-D-galactosyluronic residues. There are three pectic polysaccharides, these are homogalacturonan, rhamnogalacturonan-I and substituted galacturonans, isolated from primary plant cell walls. Homogalacturonan (HG) is a linear chain of 1,4-linked α-D-galactosyluronic residues, there are some of the carboxyl groups are methyl esterified. At the

C-2 and C-3 positions they may be acetylated. Homogalacturonans have been isolated from sunflower heads and apple pectin but the extraction treatments were likely to cleave covalent bonds, that's why it released from a heterogeneous pecticpolysaccharide (Sharma *et al*.,2006).

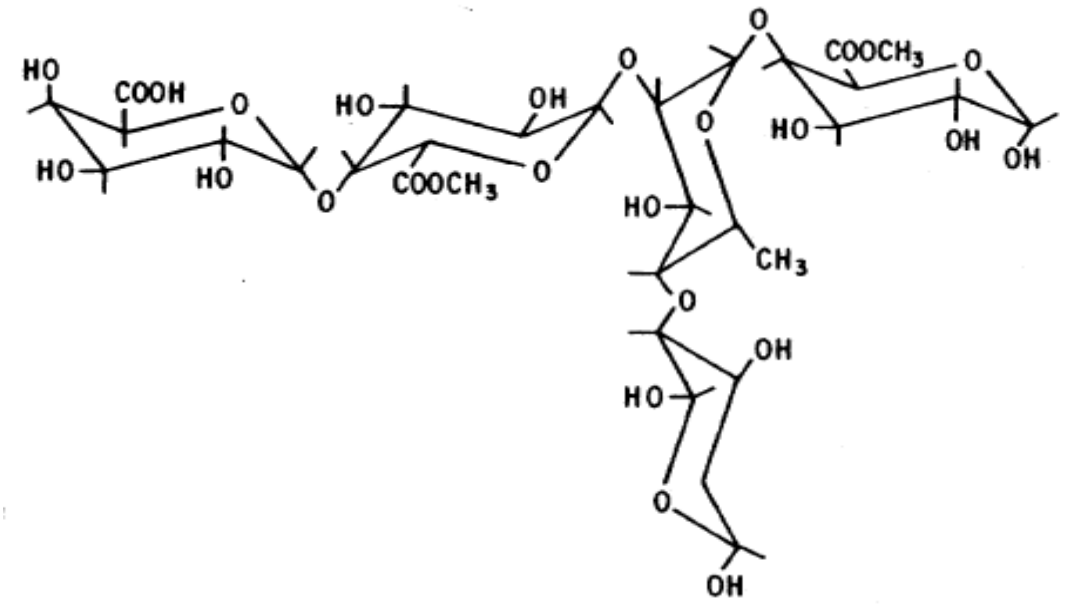


Figure 2.1: Structure of pectin

Commercial pectins are formed by a specific group of carbohydrate polymers which are composed of large backbone of linked D-galacturonic acid units, in that many of them are esterified with methyl alcohol at the carboxylic acid, interspersed with a few L-rhamnose residues linked to neutral arabinogalactan side chains. The most important application of pectin is in jellies with high sugar content, but it is also used in the pharmaceutical, dental and cosmetic industries for its jelling properties (Endress,1991).

2.2.2 Functional group

Pectins carries some non-sugar substituents, like methanol, acetic acid, phenolic acids and sometimes amide groups. Esterification of galacturonic acid residues with methanol is a very important structural characteristic of pectic substances. The definition of degree of methylation (DM) is the percentage of carbonyl groups esterified with methanol. Degree of methylation (DM) are two types, these are high-methoxy pectin (HM) and low-methoxy pectin (LM). More than 50% of the carboxyl groups are methylated and this pectins are called

high-methoxy pectins (HM), on the other hand less than that degree of methylation are called low methoxy (LM) pectins. This same condition is applicable to acetylation. Acetyl groups can be found in the 'hairy' rhamnogalacturonan regions and only present in very low amount in homogalacturonan from apple and citrus. It is found in higher amounts in homogalacturonan from sugar beet and potato (Sharma *et al*.,2006).

2.3 TYPES OF PECTIN

Based on formation of gel in the presence of divalent cations, sugar or acid, pectins can be classified into two groups:

2.3.1 Low methoxy pectin (LM)

LM pectin form gel in the presence of divalent cations, usually calcium. Gelation is caused by the formation of intermolecular junction zones between homogalacturonic regions of different chains. The structure of such a junction zone is generally known as “egg box” binding process. Initial strong association of two polymers into a dimer form weak inter dimer aggregation, mainly regulated by electrostatic interactions. Gelation ability of LM pectin increases with decreasing degree of methylation. The presence of acetyl groups in pectin is very useful because it prevents gel formation with calcium ions and gives the pectin emulsion stabilizing properties (Ahmmed, 2013).

2.3.2 High methoxy pectin (HM)

HM pectins can form gel with sugar and acid. This kind of gel is considered a 2-dimensional network of pectin molecules in which the water with sugar and acid are immobilized. The formation of the 3D network is based on the structure of junction zones in which there are chain associations stabilized by hydrogen bonding between un-dissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters. The gelation mechanism of pectins is mainly followed by their degree of esterification (DE). The attraction of pectin chains towards calcium is known to increase with decreasing degree of esterification, and with increasing polymer concentration. The influence of the charge density in polygalacturonate chain, and the distribution pattern of free and esterified carboxyl groups has an important effect on the strength of calcium binding. Molecular weight of pectin varies with plant source, raw material and extraction conditions but molecular weight determination is a challenge because of heterogeneity and aggregation which can be mist data gathering

(Ahmmed, 2013). Some factors are responsible for the conditions of gel formation and the gel strength achieved. The major role of pectin molecules is that their chain length, and the chemical nature in the junction zones. At same conditions gel strengths increase with the molecular weight of the pectin, and any treatment de-polymerization of the pectin chains is reflected in weaker gel (Sharma & Naresh, 2006).

2.4 SOURCES OF PECTIN

Most commonly pectin present in most of the plant tissues as a layer in the middle lamella and as a thickening on the cell wall. Pectin from different sources may be used for the commercial manufacture of pectin but in a limited amount. On the other hand, the pectin from different sources does not have the same gelling ability due to variations in molecular size (Simpson *et al*.,1984). Now a days, commercially acceptable pectins source are citrus peel and apple pomace. They produce different quality pectins, which are used for specific applications (May, 1990).

2.5 USES OF PECTIN

Pectin can be used in many ways. It is used mainly in foods as a gelling agent, thickener, texturizer, emulsifier, and stabilizer. Recently, pectin has been used in low-calorie foods as a fat or sugar replacer. Different uses of pectin in food and other industries are discussed here:

2.5.1 Jams and jellies

The major food items in which large amount of pectins are used are jam and jelly. Jam preparation requires brief cooking of the fruit to liberate juice and pectin through conversion of proto pectin to soluble pectin. Depending upon the conditions, additional pectins may be added at any point during preparation. Pectin is added as a dry powder mixed with sugar in a solution (Towel *et al.,* 1959).

2.5.2 Conserves

Conserves are products that do not contain a sweetener but fruit juice or fruit concentrate contain sweetener. That’s why, their soluble solid contents are lower than the products containing sweetener. As they do not contain any added sugar, They highly preferable by consumers. The total soluble solid content of conserves is 55 to 62%. At the upper level, a

rapid-set HM pectin is used, while at the lower level, a LM pectin is added to give the desired mouth feel and body of the products (Towel *et al.,* 1959).

2.5.3 Bakers' jellies

Pectin is used to make jellies that are applied to prepare bakery products. HM pectins are thermally stable, is used to make jellies that are placed in the batter or dough and baked product. Fiber entanglements will further emphasize the gel structure if the fiber content is increased. LM pectin has a wide application in bakery jam and jelly production. The use of LM pectin use requires a large amount of pectin in the formula, compared with HM pectin, to the exact firmness (Hoefler, 1991).

2.5.4 Confectionery products

Different flavored candies are produced using HM pectin. Artificial cherries can be made using pectin, where a synthetic medium is produce to control setting conditions (Peschardt, 1956)**.**Pectin is also used in edible coatings for inhibiting lipid migration in confectionery products (Brake *et al.,* 1993).

2.5.5 Frozen barriers

Pectin is used in frozen foods to prevent crystal growth, syrup during thawing, and to improve shape (Buren, 1983). Ice-cream factories use pectin for ice-cream production. They use LM pectins to improve the texture and quality of ice creams (Decker, 1951). Pectin helps to improve the texture of frozen foods by controlling the ice crystal size in them. Pectin is also used in the different gelled pudding desserts, where the mixing of fruit syrup containing pectin with cold milk. This types of dessert can be prepared without refrigeration because of the use of pectin (Hoefler, 1991).

2.5.6 Beverages

In recent years, fruit juice does not use the added sweeteners like sucrose, high fructose corn syrup or both. That’s why certain mouth feel is not present in the conventional soft drinks. The loss of mouth feel can be restored by the addition of HM pectin 0.05 to 0.10%. Pectin can also be used as a beverage-clouding agent (Hoefler, 1991; El-Shamei and El-Zoghbi, 1994).

2.5.7 Barbecue sauce

LM is added to the barbecue sauces due to its flavor release attributes and texture. The LM pectin and calcium content in the mixture determines the product's final consistency and texture (Hoefler, 1991).

2.5.8 Pharmaceutical uses

Food pectin is also used in pharmaceutical industry. Pectin influences cholesterol levels in blood and act against toxic cations. It is effective in removing lead and mercury from the gastrointestinal organs and respiratory tracts (Kohn,1982). When pectin is injected intravenously, it reduces the coagulation time of drawn blood, thus it is useful in controlling hemorrhage or local bleeding (Joseph,1956). On the other hand, pectin sulfate prolongs clotting time and can be used in place of heparin (Ramaswany *et al.*,1992).

For the treatment of iron deficiency anemia degraded pectin iron is used. Pectin has been reported to reduce blood cholesterol in a wide variety of subjects (Cedra *et al*., 1988). Cholesterol reduction in blood requires consumption of at least 6 g/day of pectin. If the amounts less than 6 g/day are not effective (Delbarre *et al*., 1977; Raymond *et al.*, 1977). The mixture of LM pectin, aluminum hydroxide, and magnesium oxide are very useful in the treatment of gastric and duodenal ulcers. Pectin alone or in combination with gelatin is used as an encapsulating agent for the preparation of medicine. HM pectin is used for the release of aspirin and act as a demulcent in minimizing the gastrointestinal (CIBA, 1967; Ashford *et al.*, 1994; Bender, 1970).

2.6 NUTRITIONAL ASPECTS OF PECTIN

2.6.1 Source of dietary fiber

Pectin is collected from plant cell walls and is analyzed as a soluble and insoluble fraction in the form of galacturonic acid after hydrolysis. Those fruits and vegetables which are rich in pectin have dietary fiber contents in the range of 1-2%. Pectin fibers has higher hydration properties than other fibers and this property is used in different food production, for example in bakery products. It has been reported that replacement of flour with citrus fibers, apple flakes and concentrates in bakery and confectionery products had a positive sensory effect. Adsorbent and bulk-forming properties of pectin have been promoted in some multi-ingredient anti constipation and anti diarrhoeal preparations.

2.6.2 Mineral binding

Dietary fiber can absorb and exchange mineral and ion. Pectin has the ability to associate ions. Due to high content of negative charges and calcium binding pectin has the ability to associate with ions.

2.6.3 Prebiotic effect

Another functionality of pectin is in prebiotic effect on human body. Pectin fermentation takes place in the large intestine by the action of bacteria. Pectin substituents are fermented in the colon by the formation of short- chain fatty acids. It has been reported that non-methyl-esterified pectins were more rapidly fermented than methyl-esterified pectins. The end products of fermentation of pectin are the short-chain fatty acids, acetate, propionate and butyrate, as well as hydrogen and carbon dioxide. The short-chain fatty acids escape colonic metabolism and transported via the portal circulation to the liver where they undergo metabolism. In the liver, they enter the systemic circulation and are distributed to the various tissues of the body.

2.6.4 Cholesterol regulation

Cholesterol regulation by pectin depends on the viscosity of pectin. Preparations of pectin with high viscosity appear to be more effective in lowering cholesterol than lower viscosity. High viscosity of pectin lower cholesterol levels by raising the excretion of fecal bile acids and neutral sterols. High-viscosity pectin may incorporate with the formation of micelle and lower the diffusion rate of bile acid and cholesterol-containing micelles through the bolus, consequently diminishing the uptake of cholesterol and bile acids. Pectin has also favorable effects on lipids.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

Extraction of pectin from different fruit peels and apple are described in detail.

3.1. RAW MATERIAL

Ananas comosus (Pineapple) and *Passiflora edulis* (Passion Fruit) peels were collected from bakeries and cool bars nearby KCAET campus and *Artocarpus heterophyllus* (Jack Fruit) peel were collected from food processing unit of KCAET for the qualitative and quantitative analysis of pectin and for determining other properties such as gelling ability, color etc. For the comparison purpose *Malus domestica* (Apple) were procured from the Kuttipuram market.

3.2. CHEMICALS AND REAGENTS

The chemicals and reagents used for pectin extraction include: Citric acid, Alcohol, Sodium chloride, Hydrochloric acid, Sodium hydroxide (NaOH) and Phenol red indicator.

3.3 EQUIPMENTS

1. Water bath
2. pH meter
3. Magnetic stirrer
4. Rotary vacuum evaporator
5. Filter paper and muslin cloth
6. Beaker
7. Measuring cylinder
8. Pipette
9. Conical flask
10. Cabinet dryer
11. Knife
12. Round bottom flask
13. Weighing balance
14. Colorimeter
15. Burette
16. Grinder

3.4 EXPERIMENTAL PROCEDURE

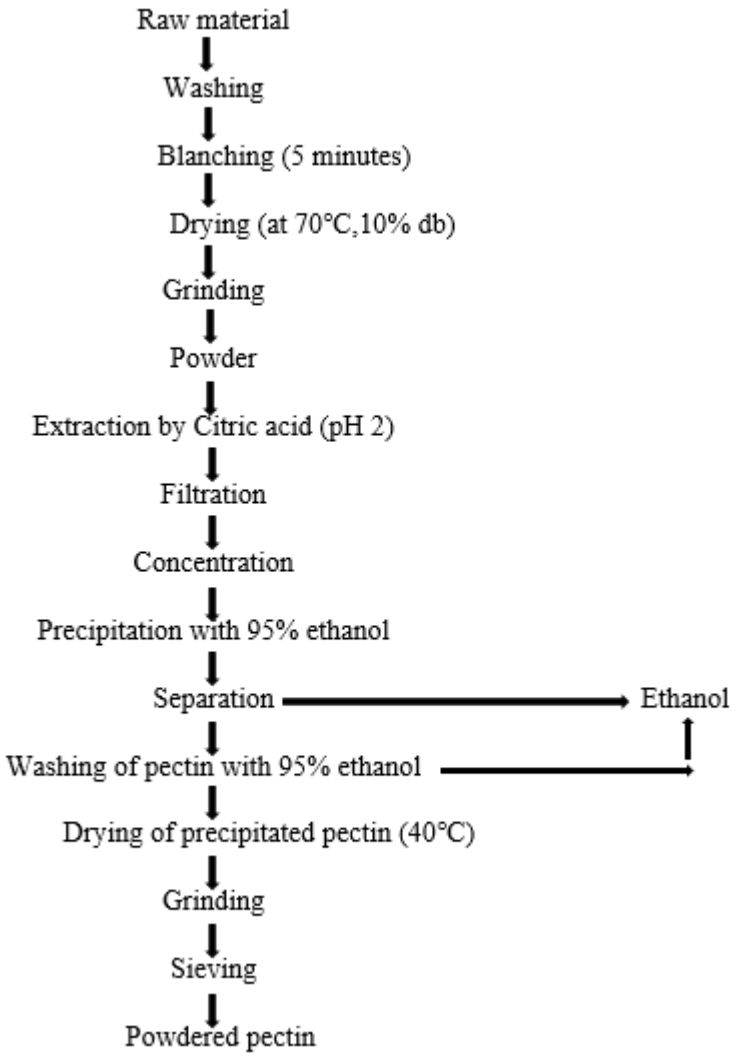


Fig 3.1. Flow diagram of pectin extraction

3.5 SAMPLE PREPARATION

The fruit peels and apples were first washed thoroughly with distilled water and chopped to bits using a knife.

3.5.1 Blanching:

All samples were plunged into boiling water for about five minutes and immersed in cold water.

3.5.2 Drying:

Blanched samples were weighed and transfer to cabinet drier and dried until the moisture content reaches to 10% (dry basis) approximately. The dried samples were then milled to powder using a blender. Then kept in tightly closed plastic bags until it used.

$$\text{Moisture content (dry basis)} = \frac{(\text{Wet weight} - \text{Dry weight}) \times 100}{\text{Dry weight}}$$

3.5.3 Grinding

After drying each of the samples were milled separately.

3.6 EXTRACTION BY CITRIC ACID

Citric acid solution having pH 2 were prepared by 10g citric acid pellets in 1000ml distilled water. About 500g of ground powder was further placed in a beaker and the citric acid was added until the sample were fully immersed. The beaker placed in the water bath at 90°C for 1 hour. After one hour of continuous stirring the mixture was cooled to room temperature and filtered using muslin cloth previously rinsed in distilled water. The retentate was again subjected to acid extraction and the whole filtrate were used for further processing.

Fig 3.2. Continuous stirring

3.7 CONCENTRATION

Four liters of filtrate obtained after citric acid extraction were concentrated to 1 liter by open pan heating.

3.8 PRECIPITATION WITH ETHANOL

The filtered extract was coagulated using 95% of ethanol added at intervals with constant stirring using a magnetic stirrer until precipitation was complete.

Fig 3.3. Precipitation

3.9 SEPARATION

The precipitate was kept aside and filtered through Whatman No.1 filter paper and then it was washed thrice with ethanol.

Fig 3.4. Filtration

3.10 DRYING OF PECTIN

The resulting material was placed in a cabinet dryer for 24h at 40°C. After drying the dried pectin was grinded into fine powders by using power driven grinder. The dried pectin powder was filled in air-tight PET bottles for further analysis.

3.11 QUANTITATIVE ANALYSIS

3.11.1 Determination of pectin yield

$$\%\text{Yield of pectin} = \frac{\text{Weight of dried pectin (g)} \times 100}{\text{Weight of sample (g)}}$$

3.11.2 Determination of equivalent Weight

Pectin (0.5g), ethanol (5mL), sodium chloride (1.0g), carbon dioxide free distilled water (100mL), and six drops of phenol red indicator were dissolved and titrated against standard 0.1M NaOH until the color of indicator changed to pink and persisted for at least 30s.

$$\text{Equivalent weight} = \frac{\text{Weight of sample (g)} \times 1000}{\text{Volume of alkali (ml)} \times \text{Normality of alkali}}$$

3.11.3 Determination of methoxyl Content

To the neutral solution titrated for equivalent weight containing 0.5g of pectic substance, added 25mL of 0.25M NaOH, shaken thoroughly, and allowed to stand for 30min at room temperature in a stoppered flask. HCl (25mL, 0.25M) was added and titrated with 0.1M NaOH to the same end point as before.

$$\text{Methoxyl content} = \frac{\text{Volume of alkali (ml)} \times \text{Normality of alkali} \times 31 \times 100}{\text{Weight of sample (g)} \times 1000}$$

3.11.4 Determination of Anhydrouronic acid content (AUA)

Total AUA of pectin was obtained by the following formula

$$\%\ \text{of AUA} = \frac{176 \times 0.1\ (Z + Y)}{w \times 10}$$

Where;

Z = ml of NaOH from equivalent weight determination.

Y = ml of NaOH from methoxyl content determination, w = weight of sample.

3.11.5 Determination of degree of esterification (DE)

The DE of pectin was measured on the basis methoxyl and AUA content and calculated by flowing formula:

$$\% \text{ DE} = \frac{176 \times \% \, MeO \times 100}{31 \times \% \, AUA}$$

3.11.6 Jelly Grade

Jelly grade was measured following test jelly method under standard conditions (TSS 68˚B, pH 3.2). The sugar, citric acid, and water were kept constant while pectin content was varied. The quality of the gel was judged and jelly grade was calculated for the well set and firm gel.

$$\text{Jelly grade} = \frac{\text{Weight of sugar (g)}}{\text{Weight of pectin (g)}}$$

3.12 QUALITATIVE ANALYSIS

3.12.1 Color characteristics

Color of product is an important parameter that will be valued during product marketing. Color of the pectin powders were measured using Hunter lab color flex meter (Hunter Associates Laboratory, Reston, Virgina, USA). The color was measured by using CIELAB scale at 100 observers at D65 illuminant. It works on the principle of focusing the light and measuring the energy reflected from the sample across the entire visible spectrum. The three-dimensional scale L*, a* and b* values were used for color measurement. The luminance (L*) forms the vertical axis, which indicates light-dark spectrum with a range from 0 (black) to 100 (white). In the same way, a* indicates the green – red spectrum with a range of -60 (green) to +60 (red) and b* indicates the blue-yellow spectrum with a range from -60 (blue) to +60 (yellow) dimensions respectively (Reddy *et al*., 2014). The instrument was standardized before placing the sample by placing black and white tile provided with the instrument. Once the instrument was standardized, it was ready to measure the color. It can also be cross checked by placing the white tile which was provided by the L*, a* and b* values. The samples were filled in the sample cup. The deviation of the color of the sample to

standard was also observed and recorded in the computer interface. The experiment was repeated thrice for each sample and average was taken as color range.

3.12.2 Solubility of Dry Pectin in Cold and Hot Water

A 0.25g of the pectin samples were separately placed in two conical flasks, followed by addition of 10 mL of 95% ethanol and 50 mL of distilled water. The mixture in the second flask was shaken vigorously to form a suspension which was then heated at 85-95°C for 15 min.

Fig 3.5: pH meter

Fig 3.6 : **Colorimeter**

Fig 3.7: Magnetic stirrer

Fig 3.8: Cabinet dryer

RESULTS AND DISCUSSIONS

CHAPTER 4

RESULTS AND DISCUSSIONS

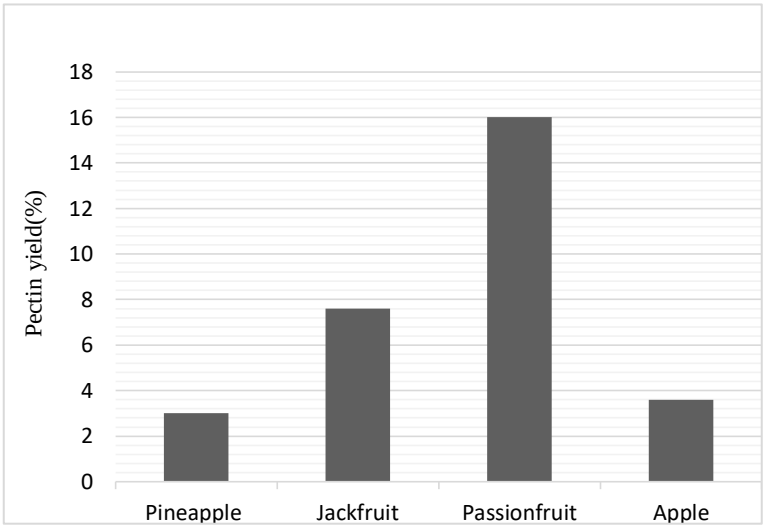
4.1 YIELD OF PECTIN

Recovery of pectin from four different samples were different. The obtained pectin yield percentage were presented in the Table 4.1

Table 4.1: Percent yield of pectin from different samples

Sample	Yield (%)
Pineapple	3
Jackfruit	7.6
Passionfruit	16
Apple	3.6

As shown in the Table.4.1 the yield of pectin extracted from passion fruit was higher than the rest of samples (16%). When compared with apple (3.6%), yield of pectin from jackfruit peel (7.6%) and passion fruit peel was higher. The lowest pectin yield was obtained from pineapple peel (3%).



Graph 4.1 : Pectin yield from different samples

Fig.4.1: Pineapple pectin

Fig.4.2: Jackfruit pectin

Fig.4.3: Passion fruit pectin

Fig.4.4: Apple pectin

Fig.4.5: Commercial pectin

4.2 EQUIVALENT WEIGHT

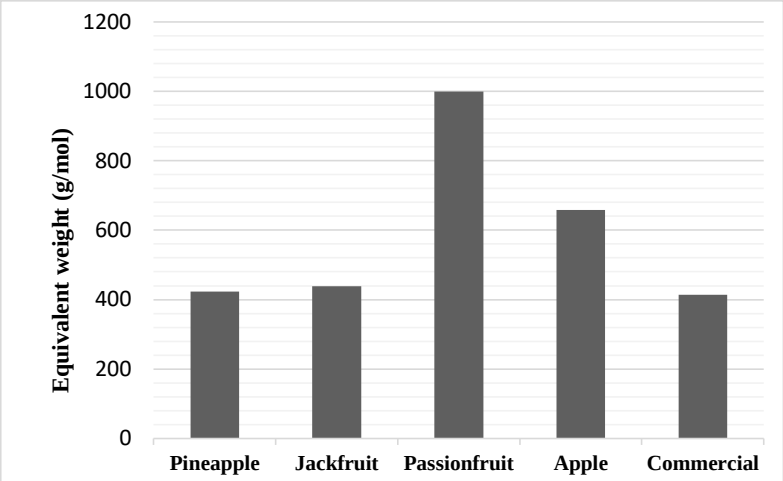
Equivalent weight is a very important physical property of pectin. It is the most important characteristic in determining the functional behavior of pectin. Gelling abilities of individual pectins are tied very closely with equivalent weight. The equivalent weight of extracted pectin was 423.7 g/mol in pineapple pectin which was near to 435.2 g/mol (Piroj *et al*). The equivalent weight of apple pectin was 657.9 g/mol, which was related to 652.48 g/mol (Sogi *et al*.,2016) and equivalent weight of passionfruit pectin (1000 g/mol) was also near to the reported value, 1180 g/mol (Florencia *et al*,2016).Lastly, the equivalent weight of jackfruit pectin was 438.6 g/mol and the reported value was 460.63 g/mol (Sarwar *et al*.,2017).

The acid used for the extraction of pectin from different fruit peels are citric acid. The citric acid was best for the extraction of pectin. The results shows that this acid type strongly influences the macromolecular and gelling properties of isolated pectin, with citric acid being the least degrading (depolymerizing and de-esterifying) extracting agent it mean the pectin isolates with the best gelling properties (Dalia *et al,* 2014).

. **Table 4.2: Equivalent weight of pectin from different samples**

Sample	Equivalent weight (g/mol)
Pineapple	423.7
Jack fruit	438.6
Passionfruit	1000
Apple	657.9
Commercial	413.2

This parameter as reported in literature varies in a wide range depending on the method and the nature of the fruits used for extraction. High equivalent weight would have higher gel forming effect. The lower equivalent weight could be higher partial degradation of pectin. The increased or decreased of the equivalent weight might be also dependent upon the amount of free acid.



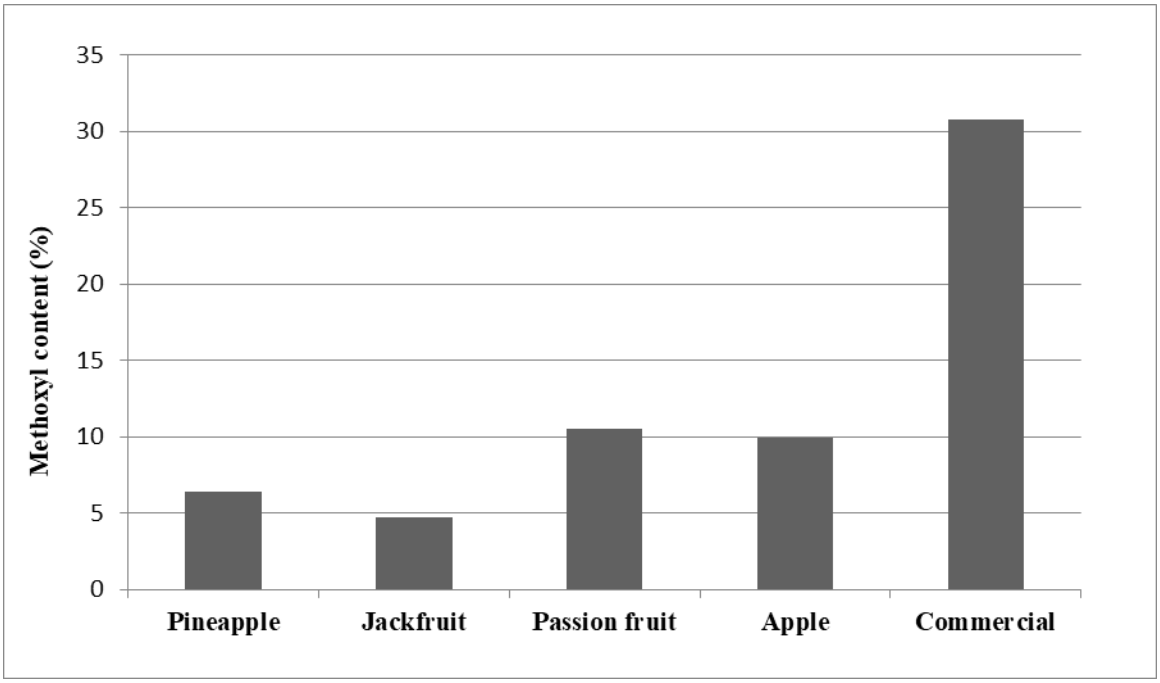
Graph 4.2: Equivalent weight of different samples

4.3 METHOXYL CONTENT

Methoxyl content is an important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the methyl ester in ratio to all esterified groups. Methoxyl content is an important factor in controlling the setting time of pectins and the ability of the pectin to form gels. Methoxyl content for extracted pectin was found to be 6.4 % (Pineapple), 4.7% (Jack fruit), 10.5% (Passionfruit) and 9.92% (Apple). Spreading quality and sugar binding capacity of pectin are increased with increase Methoxyl content. Low methoxyl pectin refers to the pectin with degree of methylation less than 50%. Which usually contain Ca ions and gels with less solids. High methoxyl pectin is the form of pectin which has a degree of methylation above 50%. It is traditionally used for canning applications. It requires high amount of sugar to gel and is very sensitive to acidity. Low methoxyl pectin has been used in the food industries to create low sugar jams because it does not require high sugar levels to gel.

Table 4.3: Methoxyl content of different samples

Sample	Methoxyl content (%)
Pineapple	6.4
Jack fruit	4.7
Passionfruit	10.5
Apple	9.92
Commercial	30.8



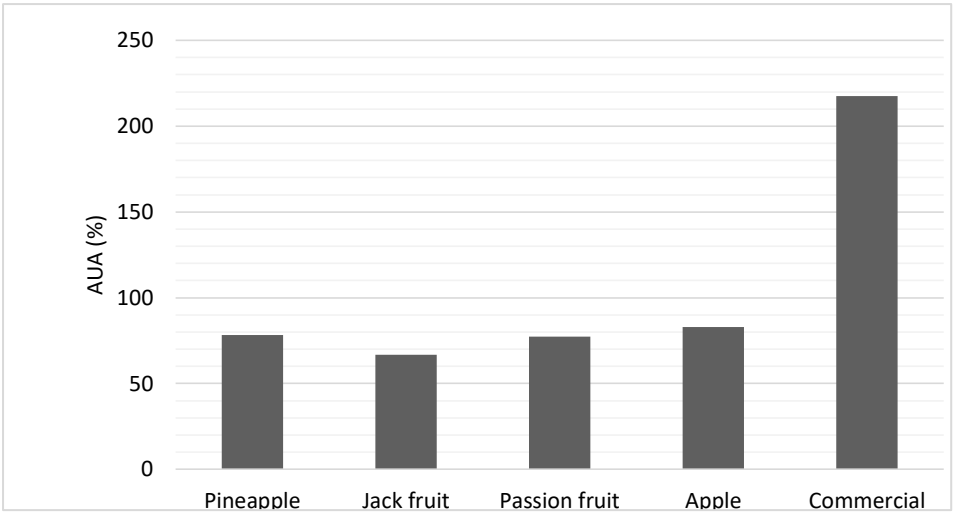
Graph 4.3: Methoxyl content of different samples

4.4 ANHYDROURONIC ACID CONTENT (AUA)

AUA is important to the gelling capabilities of given pectin. The AUA indicates the purity of the extracted pectin and its value should not be less than 65%. The value of AUA in the given sample of extracted pectin was found to be 78.14% (Pineapple), 66.9% (Jack fruit), 77.44% (Passionfruit) and 83% (Apple). Low value of AUA means that the extracted pectin might have a high amount of protein.

Table 4.4: **Anhydrouronic acid of different samples**

Sample	AUA (%)
Pineapple	78.14
Jack fruit	66.9
Passionfruit	77.44
Apple	83
Commercial	217.5



Graph 4.4: Anhydrouronic acid of different sample

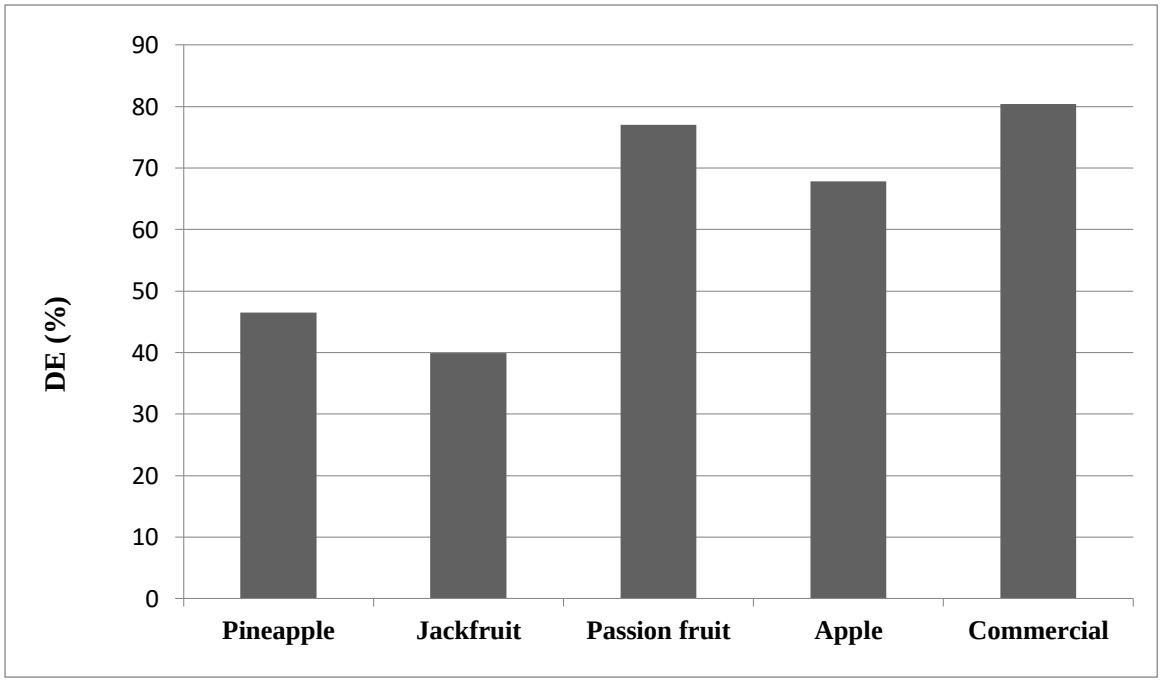
4.5 DEGREE OF ESTERIFICATION

DE is an important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the methyl ester. The degree of esterification for extracted pectin from various samples were found to be 46.5% (Pineapple), 39.89% (Jack fruit), 77% (Passionfruit) and 67.8% (Apple).

Table 4.5: Degree of esterification (DE)

Sample	DE (%)
Pineapple	46.5
Jack fruit	39.89
Passionfruit	77
Apple	67.8
Commercial	80.4

In this study, the pectin can be categorized as high methoxyl pectin (HMP) because it has a DE that is higher than 50%. Degree of esterification decreased with the increase of maturity. DE actually depends on species, tissue and stages of maturity.



Graph 4.5: Degree of Esterification of different samples

4.6 JELLY GRADE

Jelly grade is the most important parameter, which indicates the number of parts of sugar required for one part of pectin to produce jelly of desirable consistency under standard conditions (TS 68%, pH 3.2). The jelly grade of extracted pectin from passion fruit peel was 450 and that of commercial sample was 150.

Table 4.6: Jelly grade

Sample	Amount of sugar (g)	Amount of pectin (g)	Jelly grade
Pineapple	45	0.8	56.25
Jack fruit	45	0.4	112.5
Passion fruit	45	0.1	450
Apple	45	0.3	150
Commercial	45	0.3	150

Fig. 4.6. Pineapple pectin jelly

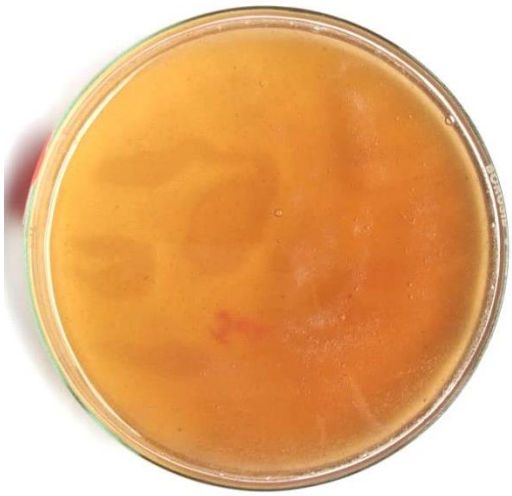


Fig. 4.7. Jackfruit pectin jelly

Fig.4.8: Passion fruit jelly

Fig.4.9. Apple pectin jelly

Fig.4.10: Commercial pectin jelly

Fig.4.11: Control jelly

4.7 COLOUR CHARACTERISTICS

Colour of product is an important parameter that will be valued during product marketing. Colour of the pectin from different sources were measured by Hunter lab colour flex meter (Hunter Associates Laboratory, Reston, Virgina, USA).

Table 4.7: color characteristics

Colour parameters	Pineapple	Jack fruit	Passion fruit	Apple	Commercial pectin
L*	36.183	39.496	51.79	34.496	82.69
a*	5.45	11.31	5.77	11.496	3.44
b*	15.85	19.02	17.12	19.076	15.31

The values indicated the L* value of passion fruit pectin were significantly more (L*>50) when compared to the other pectins. The high a* & b* value and low L* value of jack fruit and apple pectin result in dark reddish color. Even though the pineapple pectin have low a* & b* value, it have a dark shade because of low L* value (Pankaj *et al*. 2013). Incase of commercial pectin the L* value is near to 100 and a* & b* values are very small this result in white colour of pectin.

4.8 SOLUBILITY OF DRY PECTIN IN COLD AND HOT WATER

The solubility of dry pectin was observed in cold and hot water, given in the following table

Table 4.8: Solubility of dry pectin in cold and hot water

Parameter	Pineapple pectin	Jack fruit pectin	Passion fruit pectin	Apple pectin	Commercial pectin
Solubility of dry pectin in cold water	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Solubility of dry pectin in hot water	Soluble	Soluble	Soluble	Soluble	Soluble

4.9 COST ANALYSIS

Estimation of Cost of Production of pectin extraction from fruit wastes

Capacity of the cabinet dryer	=	10-15 kg
Working hour for fruit peel dry	=	10 h
Working hour for pectin dry	=	4 h
Cost of water bath (B)	=	Rs. 4,800/-
Cost of the cabinet dryer (C)	=	Rs. 50,000/-
Cost of the grinder (G)	=	Rs. 3,000/-
Cost of the induction cooktop (I)	=	Rs. 2,000/-
Cost of the rotary vacuum evaporator	=	Rs. 85,000/-
Life span of the unit (n)	=	15 years
Annual usage (A)	=	300 days
Interest rate (i)	=	11 % per annum

I. Fixed cost per year

A) Fixed cost of the water bath (B) = $\frac{i(i+1)^n}{(i+1)^n+1} \times B$

= $\frac{0.11(0.11+1)^{15}}{(0.11+1)^{15}+1} \times 4{,}800$

= Rs. 437/-

B) Fixed cost of the cabinet dryer unit (C) = $\frac{i(i+1)^n}{(i+1)^n+1} \times C$

= $\frac{0.11(0.11+1)^{15}}{(0.11+1)^{15}+1} \times 50{,}000$

= Rs. 4,549/-

C)Fixed cost of the grinder (G) = $\frac{i(i+1)^n}{(i+1)^n+1} \times G$

= $\frac{0.11(0.11+1)^{15}}{(0.11+1)^{15}+1} \times 3{,}000$

= Rs.273/-

D) Fixed cost of the induction cooktop (I) = $\frac{i(i+1)^n}{(i+1)^n+1} \times I$

= $\frac{0.11(0.11+1)^{15}}{(0.11+1)^{15}+1} \times 2{,}000$

= Rs. 182/-

E) Fixed cost of the rotary vacuum evaporator (E) = $\frac{i(i+1)^n}{(i+1)^n+1} \times E$

= $\frac{0.11(0.11+1)^{15}}{(0.11+1)^{15}+1} \times 85{,}000$

= Rs. 7734/-

Total fixed cost/year = A+ B + C + D + E

= 437 + 4549 + 273 + 182 + 7734

= Rs. 13175/-

II. Variable cost per year

i) Repair and maintenance of water bath = 2 % of initial cost of the water bath

$= 4{,}800 \times \frac{2}{100}$

= Rs.96/-

ii) Repair and maintenance of cabinet dryer = 2 % of initial cost of the cabinet dryer

$= 50{,}000 \times \frac{2}{100}$

= Rs. 1000/-

iii) Repairs and maintenance of grinder = 2 % of initial cost of the high shear emulsifier

$= 3{,}000 \times \frac{2}{100}$

= Rs.60/-

iv) Repairs and maintenance of induction cooktop = 2 % of initial cost of the grinder

$= 2{,}000 \text{ x } \frac{2}{100}$

= Rs. 40/-

a)Total Repair and maintenance charge = i + ii + iii + iv

= 96 + 1000 + 60 + 40

= Rs. 1196/-

b) Cost of energy

Energy requirement water bath = 1 kWh/ 10 h

Energy requirement of cabinet dryer = 5 kWh/10 h

Energy requirement grinder = 5 kWh/10 h

Energy requirement induction cooktop = 20 kWh/ 10h

Energy requirement rotary vacuum = 6.5 kWh/10h

evaporator

Energy requirement

2 fan	= 80 w/h	=	1.4 kWh / 10 h
3 light	= 120 w/h		
2 Exhaust	= 80 w/h		

Total energy requirement	=	37.5 kWh/ 10 h
Electricity charges	=	Rs. 5.85 / kWh
Electricity consumption charges	=	No. of days x Energy/day x Rate
	=	300x 37.5 x 5.85
	=	Rs. 65,812/-

c) Cost of raw materials

Cost of citric acid	=	Rs. 1500 / kg
Total quantity of citric acid required	=	20 g
Cost of citric acid used for extraction per year	=	20 x 1.5 x 300
	=	Rs. 9000 /-

e) Cost of alcohol

Cost of ethanol	=	Rs. 200 / l
Total quantity of ethanol required	=	3 l
Cost of ethanol used for extraction per year	=	3 x 200 x 300
	=	Rs. 1,80,000 /-

Total variable cost for extraction of pectin = a + b +c +d

	=	1196 + 65812 + 9000 + 1,80,000
	=	Rs.2,56,008/-
Total cost for the extraction of pectin per year (m)	=	Total fixed cost + Total variable cost
	=	13175 + 256008
	=	Rs.2,69,183/-
Total production of pectin / year (n)	=	10 × 300
	=	3000
Cost of production of one kg of pectin / year	=	$\frac{m}{n}$
	=	$\frac{269183}{3000}$
	≈	**Rs. 90 /kg**

CONCLUSION

CHAPER 5

CONCLUSION

From this study, we could say fruit wastes like passion fruit and jack fruit peels are very rich in pectin. Each year our country produces a lot of fruits which eventually leaves a large amount of waste; besides, food industries as well as pharmaceutical companies import pectin from different countries every year. If we can extract pectin in our country from fruit waste, it will be economically viable for industries and as well as eco-friendly. Pectin extracted from these wastes are quite a bit similar to commercially available pectin.

The equivalent weight, degree of esterification and methoxyl content of passion fruit peel pectin are higher than that of pineapple and jackfruit peel and apple pectin. The chemical quality of pectin obtained from passion fruit peel is comparatively better than other fruit peels and apple. On the other hand the percentage of pectin yield from the passion fruit peel is relatively higher compared to others with similar extraction process.

RECOMMENDATION AND FUTURE PERSPECTIVE

CHAPTER 6

RECOMMENDATION AND FUTURE PERSPECTIVE

Recommendations of this study are :

- Sample (Fruit peel) should be collected in a clean sampling bag
- Over ripened fruit peel cannot be used.
- During hydrolysis of peel, the pH range should be 1.2-2.5.

Future perspectives are :

- By using this method, we can achieve good quality and quantity of pectin.
- Seasonally available low cost fruits can be utilized in this way.
- Fruit peel waste will not create environmental pollution.
- It will reduce waste load in the processing industries.
- It is a low economic extraction method.

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CHAPTER 7

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ABSTRACT

Development Process Protocol for Extraction of Pectin from Different Fruit Peel

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ABSTRACT

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ABSTRACT

All fruit peels contain pectin at their cell wall and some pectin is also found in fruit pulp. Pectin is an important factor during fruit ripening, and the amount of pectin varies in different types of fruits. Pectin is widely used as a gelling agent, thickener, emulsifier and stabilizer in different food processing operations. Also, the pharmaceutical industries widely use pectin. It has been reported that, pectin lower the blood cholesterol levels and low density lipoprotein cholesterol fractions, which is beneficial for human health. It is also stated that, pectin may help decrease tumor cell formation. Chemically, it represents a polysaccharide, which is present in different amount in cell walls of all land plants. But some fruits are very rich in this component and can be used as source for commercial production. In this study, pineapple peel, passion fruit peel, jackfruit peel and apple were selected as a source of pectin. These peels were heated in a solution of distilled water and citric acid at a temperature of 90°C for 1 hour with continuous stirring. The solution was filtered out and the filtrate was treated with different low molecular weight alcohol such as ethanol to extract pectin. The precipitate was dried at 40°C in cabinet dryer. The product was characterized by the parameters such as yield, methoxyl content, anhydrouronic acid content, degree of esterification, equivalent weight, jelly grade and colour characteristics. The characteristic parameters were found to be in the same range as those shown by the product proposed in the market as ‘Pectin’. Finally, this pectin was used for the production of jelly.