# A STUDY ON THE CHEMICAL CHARACTERISTICS AND GEL FORMING PROPERTIES OF PASSION FRUIT PEEL PECTIN

BY

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Thesis submitted in partial fulfillment of the requirement for the Degree of Bachelor of Science in Food Science and Technology of the Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Buttala, Sri Lanka.

December, 2000

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# DECLARATION

I carried out the work described in this thesis at the Industrial Technology Institute and the Faculty of Applied Sciences under the supervision of Dr.Jaanaki Goonaratna and Dr.D.Mahinda Wickramaratne. A report on this has not been submitted to any other University for another degree.

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AFFECTIONATELY DEDICATED TO

MY EVER LOVING

PARENTS

# ACKNOWLEDGEMENT

This study was undertaken at the Industrial Technology Institute (ITI), 363, Bauddhaloka Mawatha, Colombo-07, under the supervision of Dr.Jaanaki Gooneratne, Senior Research Officer, Agro-Food Department, Industrial Technology Institute. First and foremost, I express my gratitude and sincere thanks to my external supervisor Dr.Jaanaki Gooneratne, for giving me an opportunity to undertake the study in the Agro-Food Department, and for her invaluable guidance, constructive suggestions, and kind support throughout the study period. Her valuable criticism in the preparation of the research report is well appreciated.

I would like to express my sincere appreciation to Mr.K.Nadarajah, former Head, Department of Natural Resources, and Mrs.Indira Wickramasinghe, Lecturer, Department of Natural Resources, Faculty of Applied Sciences, Sabaragamuva University of Sri Lanka, Buttala, for providing me this opportunity to carry out this study at the Industrial Technology Institute, Colombo-07.

I am indebted with my deep sense of gratitude to my internal supervisor Dr.D.B.M.Wickramaratne, Head of the Dept.of Physical Science, Faculty of Applied Sciences, Sabaragamuva University of Sri Lanka, Buttala, for his constructive criticism, encouragement and valuable advice given to me throughout this study.

I would like to express my heartfelt thanks to Dr.K.K.D.S.Ranaweera, Senior lecturer, Dean of the Faculty of Applied Sciences, and Mr.M.A.Jagath Wansapala, Lecturer, Head of the Department of Natural Resources Science, Faculty of Applied Sciences, Sabaragamuva University of Sri Lanka, for facilities provided to me throughout this study.

I am specially entitled to express my thanks to Ms.Theja Herath, Ms.Medha Gunatillake, Ms.Thushara Pelwatte and Ms.Sriyani Kumari and other members of the staff at the Agro-Food Technology Section, ITI, who helped in numerous ways to make this project successfully. I owe my deepest thanks to Ms.Kawshalya Ponnampalam who helped me to computerise this report. My special thanks are extended to my colleagues for their voluntary help to complete the study.

Finally but special affectionate appreciation goes to my uncle and his family members for their kind inspiration.

# ABSTRACT

Pectin, a high molecular weight, partially methylated polygalacturonic acid occurs in the middle lamella or intercellular wall of plant material. Pectin is widely used in food industry, commonly as a gelling agent in jams and jellies, as stabilisers in dairy products and frozen desserts and as a bulking agent or a fat replacer in dietetic foods. Present commercial sources of pectin are citrus peel and apple pomace.

The objectives of this study were (a) to extract and isolate pectin found in passion peel a waste product in food industry (b) to compare the gel forming properties of the passion peel pectin with citrus pectin and (c) investigate on methods for increasing the yield of pectin using surfactants.

Pectin was extracted and isolated from passion fruit peel after pre-processing, at pH 1.34 (using 1N HCl) and at 65°C, for 2 hr and filtered. The pectin was isolated by precipitating with 96% ethanol, filtering, washing with acidified ethanol and absolute ethanol and air- drying at 40-45 °C. Four different pre-processing methods used are described as follows:

Method 1 A: Fresh passion peel was blended with water (1:10 W/V).

Method 1B: The passion peel was dried at 50-55 °C and powdered to pass through mesh size 40, using a Wiley Mill. The powder was blended with water (1:20 w/v)

Method 2A: Passion peel was steamed and the endocarp was scooped out and blended with water (1:10 W/V).

Method 2B: Passion peel was steamed and the endocarp was scooped out, dried at 50-55°C, powdered and blended with water (1:20 W/V).

The yield of pectin varied from 14.5 to 29.0 % with different pre-processing methods. Results showed that Method 2B gave significantly higher yield of pectin, compared with other three methods. Passion peel pectin contains high methoxyl rapid set pectins (~ 9% of methoxyl content) with the acetyl value was 1.2 % or less. The purity of pectin was quantified as anhydrogalacturonic acid and was found to be 83%.

The gel-forming properties of pectin at 140-gel grade were of good quality, when compared with citrus pectin. Pectin extracted from Method (2A) and (2B) formed better gels than from method (1A) and (1B).
This may be attributed to the decrease in % Acetyl content on steaming.

The addition of 2.5% of a low molecular alcohol diethylene glycol to the acid extractant resulted in an increase in the pectin yield by 55-90%. It was also shown that the addition of alcohols resulted in a measurable increase in the pectin gel strength.

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## INTRODUCTION

Pectin is a linear polysaccharide, which acts as a cellular binder in the peels of many different fruits and vegetables. Chemically it is a partially methoxylated galacturonan. Waste products from the passion fruit industry, peel (approximately 46%) can be used as good source of pectin. (Prasad, 1980). Pectin is widely used in food industry, commonly as a gelling agent in jams and jellies, as stabilizers in dairy products and frozen desserts and as a bulking agent or a fat replacer in dietetic foods. Present commercial sources of pectin are citrus peel and apple pomace.

In most of the horticulturally advanced countries like USA, Australia, Brazil and Israel, 40-60 percent of total production of fruits and vegetables are processed into different products. These large-scale factories minimise the quantities of factory wastes by exploitation into value added by -products and improving the overall economics of these units (Anand and Maini, 1997).

In terms of Sri Lankan context the use of passion peel waste for extraction of pectin is both economical and of value as a by – product of the fruit processing industry.

The present study was carried out with the following objectives.

(a) To extract and isolate pectin from passion peel (waste) to produce an economically valuable by-product.
(b) To compare the gel forming properties of the passion peel pectin with citrus pectin.
(c) Investigate on methods for increasing the yield and gel forming characteristics of pectin using surfactants.

# CHAPTER- 02

## LITERATURE REVIEW

### 2.1 General Description of Passions

Passions are included in a large genus of herbaceous or woody tendril climbers, mostly distributed in tropical and sub tropical America, a few in tropical Asia, Australia and Polynesia (Deshaprabu, 1966). The commercial development for this crop took place only 60 to 70 years after *Passiflora edulis* was officially introduced into Queensland in 1861(Winks, *et.al.*1988). Many of the species are of ornamental value and a few are cultivated for their edible fruits (Deshaprabu, 1966).

### 2.2 Origin and Distribution

The edible commercial species of passion fruit originated on the edges of South America rain forests in the Amazon region of Brazil and possibly in Paraguay and Northern Argentina. The purple passion fruit (*P. edulis*) is adapted to the cooler subtropics or at high altitudes in the tropics, while the golden passionfruit (*P. edulis, P. flavicarpa*) is more suited to topical lowland conditions. Passion fruit became naturalised in most of the tropical and subtropical world, (Winks, *et.al.*1988).

### 2.3 Botany

The family *Passiflora* includes 550 species in 12 genera and is represented by more fruits species than probably any other plant family. The most important genus, *Passiflora*, has about 400 species which are mostly native to tropical America, but include about 40 in Asia, Australia and the South Pacific and one in Madagascar (Winks, *et.al.* 1988).

In most countries passion fruit production is based on cultivars of the golden passion fruit. The major exceptions are South Africa, Kenya and New Zealand where production is dependent on lines of the purple passion fruit and Australia where hybrids between the two forms are exploited.

Commercial *Passiflora* species are vigorous woody perennial vines with medium to large leaves (8 to 30 cm long) with toothed margins and usually bearing glands on the petioles. The bisexual flower is fragrant, borne solitary at least nodes of new growth, is about 4 to 5 cm in diameter and very conspicuous and colourful. It has five whitish sepals and petals and two purplish rows of thread like rays (corona) (Winks, *et. al.* 1988).

The pistil consists of an ovary and tripartite style, each branch terminating in a sticky stigma. Passion fruit species have a protandrous habit with pollen from the authors ripen before the stigmas are receptive. The parts of the flower supposedly represent the implements of the crucifixion. The fruit is round or oval, about 4 to 6 cm long and greenish-yellow or purple when ripe and smooth. It consists of a thin, shell like rind within which are numerous small black or brown seeds, each enclosed in a yellow or orange aromatic juicy pulp which has an acid but pleasant flavour (Deshaprabu, 1966).

The vines of the golden passion fruit are similar to those of the purple passion fruit, excepts that they are generally more vigorous and have larger leaves, flowers and fruit. The purple passion fruit has purple/red pigment in the stems, leaves and tendrils and has a deeper purple in the corona of the flower (Winks, *et. al.* 1988).

### 2.4 Nomenclature

Pectin is a generic term which is applied broadly for all pectin substances which are usually designated as carbohydrate derivatives with high molecular weights and a complex composition (Rouse, 1977).

#### 2.4.1 Pectic substances

Pectic substances are complex colloidal carbohydrate derivatives containing as their major structural feature acid polysaccharides composed primarily of galacturonic acid units (shown in Figure: 2.1), interspeted by rhamnose resides. Neutral sugars such as arabinose, galactose, rhamnose, xylose have been identified as associated with the pectin molecule as a side chain attachment (Stuekrath, *et.al.* 1998).

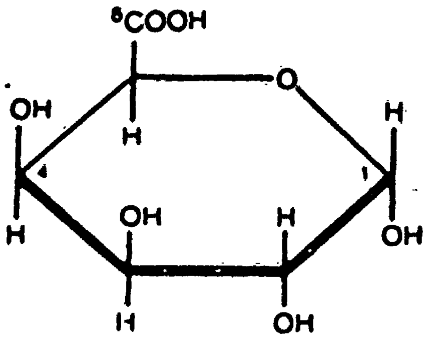


**Figure: 2.1 D-Galacturonic acid**

### 2.4.2 Protopectin

The term protopectin is applied to the water-insoluble parent pectic substance which occurs in plants and which yields upon restricted hydrolysis pectin or pectinic acids (Rouse, 1977).

### 2.4.3 Pectinic Acids

The term pectinic acid is used for colloidal polygalactronic acids containing more than a negligible proportion of methyl ester groups. Pectinic acids, under suitable conditions are capable of forming gels with sugar and acid or if suitably low in methoxyl content, with certain metallic ions. The salts of pectinic acids are either normal or acid pectinates (Rouse, 1977).

### 2.4.4 Pectin

Pectin (or pectins) designates those water soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels with sugar and acid under suitable conditions (John, 1987).

### 2.4.5 Pectic Acids

These are pectic substances, which are composed mostly of colloidal polygalacturonic acid and are essentially free from methyl ester groups. The salts of pectic acid are either normal or acid pectates (Hodge and Osman, 1976).

## 2.5 Occurrence and Functions of Pectic Substances

Pectin is the intracellular cement of cell wall tissue occurring in fruits and succulent vegetables. Pectin is produced commercially from the white *spongy albedo* (parenchymatous cells) of citrus fruits whose primary walls are said to contain a large proportion of protopectin (Kertesz, 1951).

Some important functions of the pectic substances in plants as described by Kertesz (1951) are to act as nature's cement in holding adjacent cells together, to maintain proper dehydration of young growing cells, to offer winter hardiness to plants and to provide the fruit rigidity or firmness.

## 2.6 Physical Properties of Pectin

### 2.6.1 Solubility

Water soluble, oxalate soluble and acid soluble pectic substances of high molecular weight degraded pectic substances, which do not contribute to consistency or viscosity. Water-soluble fraction includes pectin and those colloidal pectinic acids of sufficiently high methyl ester content, which are located largely in the liquid phase of a food product. They contribute to consistency and serum viscosity. In addition, they act as colloid stabilisers and play an important role in preventing the setting or flocculation of the dispersed solid phase in products such as tomato and citrus juices (Stuekrath, *et.al.*1993).

Oxalate - soluble fraction includes pectic acid and those colloidal pectinic acids of sufficiently low methyl ester content to be insoluble in water and form gels or precipitates with polyvalent metal irons. They may occur naturally in small amounts or may be formed in large amounts through the action of the enzyme, pectin methyl esterase (pectase, pectinesterase) on water-soluble pectic substances.

Acid-soluble fraction (proto pectin) is an ill-defined water-insoluble substance in plant tissue which on restricted hydrolysis (viz. warm, dilute acid) yields protopectinic acids. Over extended period of storage, slow hydrolysis of this material may cause an increase in the pectin or pectinic acid content of the product. Protopectin may act as a large insoluble water-binder and in this manner may contribute to consistency (Bender, *et.al.*1953).

### 2.6.2 Viscosity

The viscosity of a pectin solution is dependent on the degree of polymerisation of the pectin. The viscosity is also influenced by the pH of the solution' the presence of electrolytes, the temperature, the pectin concentration and the shear rate (Neukom, 1967). Viscosity measurements are usually employed to determine the molecular weight of pectins, and molecular weight by this method may range from about 50,000 to around 300,000 (Neukom, 1967).

### 2.6.3 Gel Formation

A well-known characteristic of pectin is its ability to gel formation and practical gel formation, and its regulate the consistency or body of preserves and jellies in their finished condition and an understanding of these functions is helpful in the most efficient use of pectin. The setting or gelling of preserves and jellies requires the presence of four ingredients, namely; pectin, sugar, acid and water in a definite relationship to each other in this system pectin is the gelling agent. It is the substance whose physical transformation through aggregate bonding changes a batch of syrup into a solid jelly (Anon, 1964).

Sugar and acid are the agents, which bring about this physical transformation, while water is the solvent into which the other three ingredients are dissolved. Since we have in the jelly batch variable and interdependent concentration to the role of each. Normally, the preserve or jelly batch is cooked to an established degree Brix or percent of soluble solids, which automatically eliminates sugar solids as available. Thus the fractionating of pectin in the preserve batch can be studied largely in respect to variable degrees of acidity (Kossori, *et. al.* 2000).

The carboxyl groups of the polygalactaronic acids may be partially esterified by methyl groups as in pectinic acid. It is the degree of esterification that provides the name for the high ester or high-methoxyl (HM) and distinguishes them from the low-ester or low-methoxyl (LM) pectins of commerce (John Keller, 1987).

Although both types of commercial pectins will form the pectin-sugar-acid jellies, gelation occurs under different conditions. The high methoxyl or typical commercial pectin, in order to form gels, must meet two requirements. First, the sugar content or soluble solids level must be over 50% of the weight of material in the preparation and second, the acid level must be adjusted to pH 2.9 to 3.4 depending on whether the pectin is rapid or slow setting (John Keller, 1987).

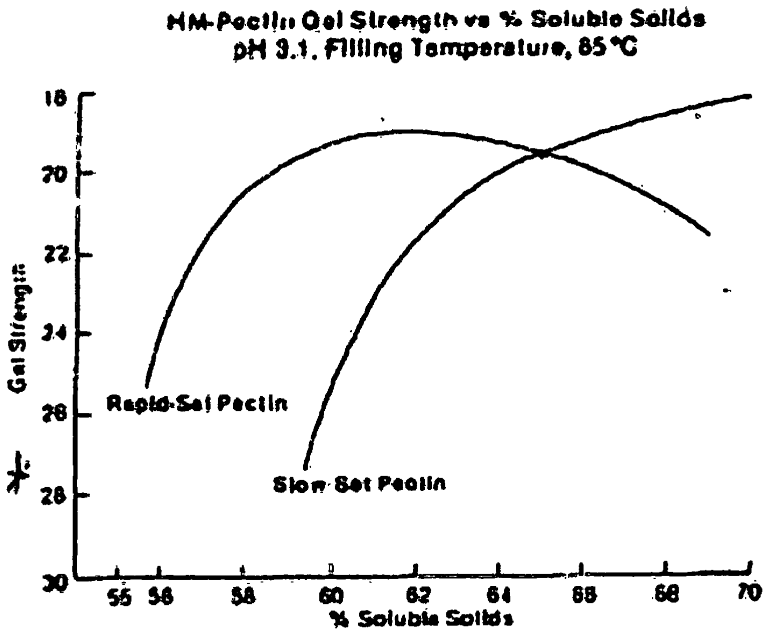


Figure:2.2 HM. Pectin Gel Strength vs % Soluble Solids pH 3.1, Filling Temperature, 85°C

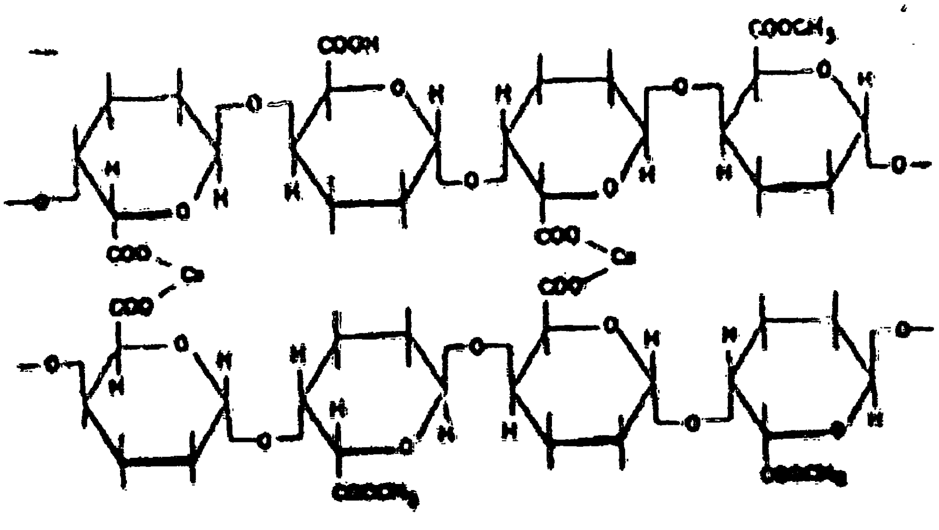


Figure:2.3 Low-Methoxyl Pectin Gel (Calcium Cross- Linking)

Low ester or low-methoxyl pectins, on the other hand, form gels when an alkaline earth cation, usually calcium, is included in the gel media. Sugar is not required in manufacturing gelled products using the low-ester pectin and these pectin forms suitable gels over a pH range of 2.5 to 6.5.

LM pectin have a wide range of food application since the soluble solids and pH limitations are greatly expanded in comparison with HM pectin. Low - Methoxyl pectin Gel (Calcium cross Linking) is shown in Figure: 2.3.

## 2.7 Chemical properties of pectin

The presence of free carboxyl groups causes pectin solutions to display an acidic pH. A one-percentage solution of non-buffered pectin (no neutralization of the carboxyl groups) may give a pH ranging from 2.7-3.0 (Nelson, *et.al.* 1976).

Insoluble pectic substances act as cation exchanges. They are very selective for Ca++ and for heavy metalions (Zn++, Cu++, Fe+++ etc). When subject to acidic conditions, the glycosidic bonds and the methyl ester linkages of pectin are prone to hydrolysis leading eventually to galacturonic acid. Hydrolysis of acid sensitive glycosidic links, eg: arabinds usually lowers the hydrolysis of acid sensitive glycosidic links, eg: arabins usually lowers the nonuronide content of acid treated pectins. Severe acid treatment leads to the decarboxylation of the carboxy group and extensive degradation. During acid treatment at reduced temperature the rate of glycosidic hydrolysis is much slower than the rate of de-esterification (Nelson, *et.al.*1976).

Therefore preparations of low ester pectins with little chain cleavage is possible (Smi and Bryant, 1968). The methyl ester groups of pectic substances are rapidly saponified by dilute alkali at room temperature. Even at this temperature some degradation of the polygalacturonic acid chain takes place, which is very strongly enhanced by a rise in temperature (Neukom, *et.al.*1960).

This pronounced alkali sensitivity of pectin is unexpected, as the common polysaccharides are usually very alkali-resistant.

Pectic substances are also degraded in the presence of oxidizing agents such as peroxide, permanganate, dichromate, free halogens and ascorbic acid. Pectins in solutions or in the plant are degraded by irradiation. Severe grinding (eg: in a ball mill) also causes a decrease in the molecular weight of pectins (Anand and Maini, 1997).

## 2.8 Sources of Pectin

Although pectins are found in the cell walls of all higher land plants, there is great variation in the pectin content of different plant varieties as well as in various plant materials (see Table 2.1) of the various plant materials. One third of the dry weight of the albedo or white portion of citrus peel and 17% of dried apple pomace is pectin. From these two sources the major portion of commercial pectin is produced today (1986).

**Table: 2.1 Approximate pectin content of various plant materials.**

| Plant material | Total pectin substance % of dry substances |
|---|---|
| Potatoes | 2.5 |
| Carrots | 10 |
| Horseradish | 15 |
| Tomatoes | 3 |
| Apples | 47 |
| Apples pomace | 15-20 |
| Sunflower heads (without seeds) | 25 |
| Sugar-beet pulp | 15-20 |
| Citrus peels | 30 |
| (albedo) | 35 |

Source: pectin substance enzyclopedia of chemical technology (1967)

## 2.9 Extraction of Pectin

The waste materials from fruit and vegetable industry are taken for extraction of pectin either when it is fresh or in dried form. There are number of methods introduced to extract pectin. Some of these are given below.

### 2.9.1 Acidic media extraction:

It is the common method (Acid hydrolysis). The waste material is mixed with sufficient quantity of acidulated water having 0.25 to 0.5 percent Hydrochloric acid or Nitric acid and extracted for about 30 to 60 min. at boiling temperature. Then liquid separated and cooled immediately and the extraction is repeated once or twice in the similar manner and all extracts combined together clarified by setting overnight and frame filler press (Ranganna, 1986).

### 2.9.2 Enzymic extraction:

Effects of various extraction techniques, involving acid hydrolysis, purified enzymic preparations if cellulases and hemicellulases on pectin from raw materials were evaluated. Pectins produced enzymatically did not form gel (Shlcodina, *et.al.* 1998).

### 2.9.3 Salting out method

This method is at temperature of 95-100°C, 100 ml of solvent, extraction time 2 hr and pH 2.1, and $Al_2(SO_4)_3$, 4g/10g peelings (Chen, 1994).

### 2.9.4 Pre-treated in an electromagnetic field of super-high frequency:

10 min. preliminary microwave heating (2450 MHz, 0.5 kW) of crushed fruit materials give a higher pectin yield. This is dependent upon material used (Kratchanova, *et.al.* 1994).

### 2.9.5 Intensified extraction:

Process involving preliminary swelling of the material for 40-45 min., follows by processing in grooved rolls. Using the pressure of 1.3-1.4 MPa, pectin yield could be increased by 30-40% (Saloov and Yusupov, 1989).

### 2.9.6 Microwave heating extraction:

Use of microwave heating to reduce the extraction time of pectin is studied. Pectin is extracted into 0.05 N HCl after heating by microwave (MWH, 2450 MHz, 1030 W, using 3 cycles of 5 min duration) or by water bath at 85°C (Manabe, *et.al.* 1988).

## 2.10 Dietary benefits of Pectin

In most animal species, including humans, the consumption of a food with a high fibre content induces a degree in the digestibility of the food and in the availability of proteins, fats and other material, vitamins and carbohydrates. Dietary fibres as defined by Trowell (1976), include not only material, such as wheat bran, but also viscous soluble polysaccharides such as pectin locust bean gum, caraganam, alginic acid and gum arabic, (Kossori, *et.al.* 2000).

The definitive studies in man using the ileostomy technique demonstrated that pectin is decomposed chiefly in the colon and that bacterial rather than animal enzymes are involved. Detailed studies carried out in the rat showed that the intestinal flora attacks apple pectin and metabolized into galacturonic acid, other organic acids that are capable of resorption and finally carbon dioxide and water. In the digestible energy of pectin was negative value. (e.g: for citrus pectin - M and pectins were reported as -1.60 and -1.65 k cal/g respectively (Viola, 1970).

Animals, which fed pectin, showed a lowering of plasma of liver cholesterol 5% below their control value. (Leveille and Saubertich, 1966). Pectin possesses other activities besides its effect on serum cholesterol, its showed that reducing of gallstone formation when compared with controls and anti diarrhoeal effect of pectin (Bender, 1959). The inflow of pectin and the subsequent return to normal pH by its digestion adversely affect the growth of the pathogens and encourage the normal flora to return to full activity (Lorne, 1978).

## 2.11 Food application of pectin

The bulk of the pectin production is utilised in the manufacture of jellies, jams, preserves, marmalades and various fruit spreads. Pectin is the only jellying agent permitted to be used in the United States for making 65% soluble solids, jellies and preserves (Rouse, 1977). The standards of identity state that pectin may be used in a quantity which normally compensate for deficiency of the natural pectin content in fruit or fruit juices (Pedersen, 1995).

### 2.11.1 Gelling agent in jams and jellites

Pectin is an important structural element of all plant foods. Chemically speaking it is a macromolecular compound belonging to the hetero polysaccharide group. Main component is polygalacturonic acid, partially esterified with methanol. When all carboxyl groups are free, i.e.unesterified; pectic acid is formed. Salts of this acid are pectates (Houska, 1996). In natural pectic acid is esterified to varying degrees with methanol. These compounds are known as pectins, themselves classified as high-esterified pectins for the rest (Kratz and Zedler, 1994).

### 2.11.2 Frozen fruits

Dry pectin mixed with sugar when added to the prevention of excess "bleeding" or loss of juice upon thawing, and improvement in the colour and a fresh appearance of the product (Rouse, 1977). The addition of pectin with a methoxyl content about 5% decreased the amount of liquid lost when added to whole strawberries, cherries, raspberries and black berries and black berries. Control of browning of frozen sliced apples is accomplished when they are dipped in a combination of pectin, sulphur dioxide and ascorbic acid (Lee *et.al.*1951).

### 2.11.3 Bakery products

Because of its water-holding capacity, pectin has been useful in bakery products to retard staling. Pectic acid in leavening powder lengthens the useful life of the mixture, controls the release of carbon dioxide and leaves no undesirable mineral residue (Kertesz, 1951). A mixture composed of 65% pectic acid, 25% sodium bicarbonate and 10% starch will evolve about 13% of the available carbon dioxide (Kratz and Zedler, 1994).

### 2.11.4 Emulsifiers and thickeners

The emulsifying properties of pectin have suggested the use of pectin in essential oil emulsions, mayonnaise, salad dressings table sauces, ice creams and for the emulsification of castor and mineral oils (Rouse, 1977). Pectin as a thickener has been added in milk beverages, cream whip aids, artificial drinks, toothpaste, cosmetics, soups and many other preparations (Endress and Rentchler, 1999).

### 2.11.5 Pectinate coatings

Pectinate films in the meat-packing industry as casing on sausages and hams are applied by dip coating or spraying. By this process the food is dipped for a few seconds into a solution containing a dispersion of calcium'sodium propionate held at a room temperature just above the gelling point and then dried in a current of air below 60°C (Schultz *et.al.* 1948).

### 2.11.6 Desserts and salad gels

Recipes procedures for low-solids fruit and vegetable juice gels, deserts, cold mix dehydrated fruit spreads and dessert gel powders have been developed with commercial products of low-methoxyl pectins (Joseph, 1955, Anon 1964, Rouse, 1977).

### 2.11.7 Confectionery

As texturing jellying and thickening agents pectins are being used increasingly in the confectionery industry because of their versatility and technological advantages (Kratz and Elder, 1994). Both high and low methoxyl pectins are used in the manufacture of confections such as gumdrops, low-sugar candies and to form protective coatings or glazing agents for candies (Rouse, 1977).

### 2.11.8 Bake proof fruit preparation

Pectins are being used increasingly in fruit preparation for baked goods, to improve baking stability and resistance to mechanical processing. Properties of pectin and their adaptation for bakery use are discussed under the headings;

I. Chemical composition and gelling behaviour of pectins, gel formation in low-esterified pectins; Gelling temperature, filling temperature; pregelling and

II. Apple pectin for bake-proof fruit preparations - strength, consistency, baking stability and surface gloss; high esterified pectins; low esterified pectins. Apple pectins are particularly suitable for use in bake-proof fruit preparations (Kratz and Kratz, 1992).

### 2.11.9 Use of pectin in dairy products

Use of pectins, particularly low methoxyl (LM) pectins, in dairy products include good consumer acceptability, good flavour release especially at acid pH, relative stability to acid pH, smooth clean controllable mouth feel, controllable setting and thickening in terms of temperature, good clarity in water systems, and gelation with naturally present Ca. (Macfadyen, 1993).

### 2.11.10 Fat replacer

The fat replacer "Slendid" (Manufactured by Hercules Inc.) is described, it is a pectin gel preparation derived from citrus peels. Characteristics of "Slendid" are discussed; advantages over other fat replacers include its neutral taste, heat stability and low calorific value. Applications considered include calorie-reduced foods, salad dressing meat products, soups, sauces, desserts, dairy products and bakery products (Pszczola, 1991).

### 2.11.11 Pharmaceutical and medicinal

Pectin processes in innumerable properties, other than those of jellying that have potential value in the medicinal field. Scraped apple peeling, with its high pectic content, has been used for years in the treatment of gastrointestinal discomfort, especially in children (Trowell, 1976). Bacterial action of pectin (metallic pectinate) was demonstrated by Haynes *et.al.* (1937). In the treatment of wounds that included osteomyelitis and other deep wounds as well as superficial ones with an aqueous solution of pectin. The pectin seemed to be non-irritating and apparently stimulated growth of the granulative tissue. Arnold (1939) presented date on the bacteriocidal action of pectin and pectinates of nickel, cobolt, manganese, lead, zinc, copper, calcium and silver. His result shows that pure pectin and calcium pectinate did not process bacteriocidal power. Other clinical evidence has demonstrated its value in acute ulcerative colitis, infected wounds, burns, postoperative dressings, athletes foot and certain chronic forms of skin diseases (Lome, 1978).

# CHAPTER- 03

## MATERIALS AND METHODS

### 3.1 Materials

### 3.1.1 Selection of passion fruit

Yellow colour passion fruit (*Passiflora indica*) peel was selected for this study. Mature, undamaged and healthy fruits were collected from the local market.

### 3.1.2 Chemicals

All the chemicals used in the study were obtained from reputed chemical firms and were analytical grade.

### 3.2 Methods

### 3.2.1 Method of pre-processing and extraction of pectin

### 3.2.1.1 Extraction of pectin from whole peel

Procedure:

A 20-g of yellow colour passion fruit peel powder was weighed into a 100-ml beaker, and 400 ml distilled water was added. The mixture was stirred to dissolve the peel powder in water. Then the pH of the mixture was measured and adjusted to 1.34 using 1N hydrochloric acid. Then the mixture was heated at 65°C for 2 hours. After heating it was filtered into an another 100-ml beaker when hot. The filtrate was cooled to room temperature and added 96% ethanol in 1:2 ratio to the filtrate. Then it was allowed to precipitate the pectin for 16 hours. After the precipitation of pectin in ethanol, the precipitate was filtered and firstly washed by acidified 70% ethanol, secondly by 70% ethanol and finally 96% ethanol and got the washed pectin. Then it was dried in an oven at 40°C until get constant weight of pectin. Finally the percentage of pectin in dried peel was calculated. Same procedure was followed to fresh peel also but the peel to water ratio was 1:10 and pectin was extracted. (Refer to Figure 3.1)

### 3.2.1.2 Extraction of pectin from peel endocarp of passion fruit

Procedure:

Passion fruit peel was washed and cut into pieces. Then it was steamed and removed the outer coat of the peel (endocarp extraction). After that pectin was extracted freshly and from dried powder. The pectin was extracted as given in Figure 3.2 as for Method 2A and 2B.

### 3.2.1.3 Pectin extraction in the presence of alcohol surfactant (Diethylene glycol)

Procedure:

A 10 ml of diethylene glycol was added to 400 ml of distilled water, heated up to $87^0$-$90^0$C in such a way that the final volume was 400 ml. Concentrated hydrochloric acid 2 ml was then added and 20g of passion peel powder was added, continuously stirring the mixture with a mechanical stirrer. The extraction was carried out at $65^0$-$70^0$C range for 2 hours, continuously stirring the mixture. The mass was filtered when hot, the filtrate was cooled down to room temperature, its volume was measured and it was coagulated by the addition of a double volume of 96% ethanol. After staying for 16 hours at room temperature the precipitate was filtered, washed twice by 70% ethanol containing hydrochloric acid, successively washed with 70% ethanol to neutral reaction and washed twice more with 96% ethanol. Then it was dried in oven at $40^0$C to a constant weight (Refer to Figure 3.3).

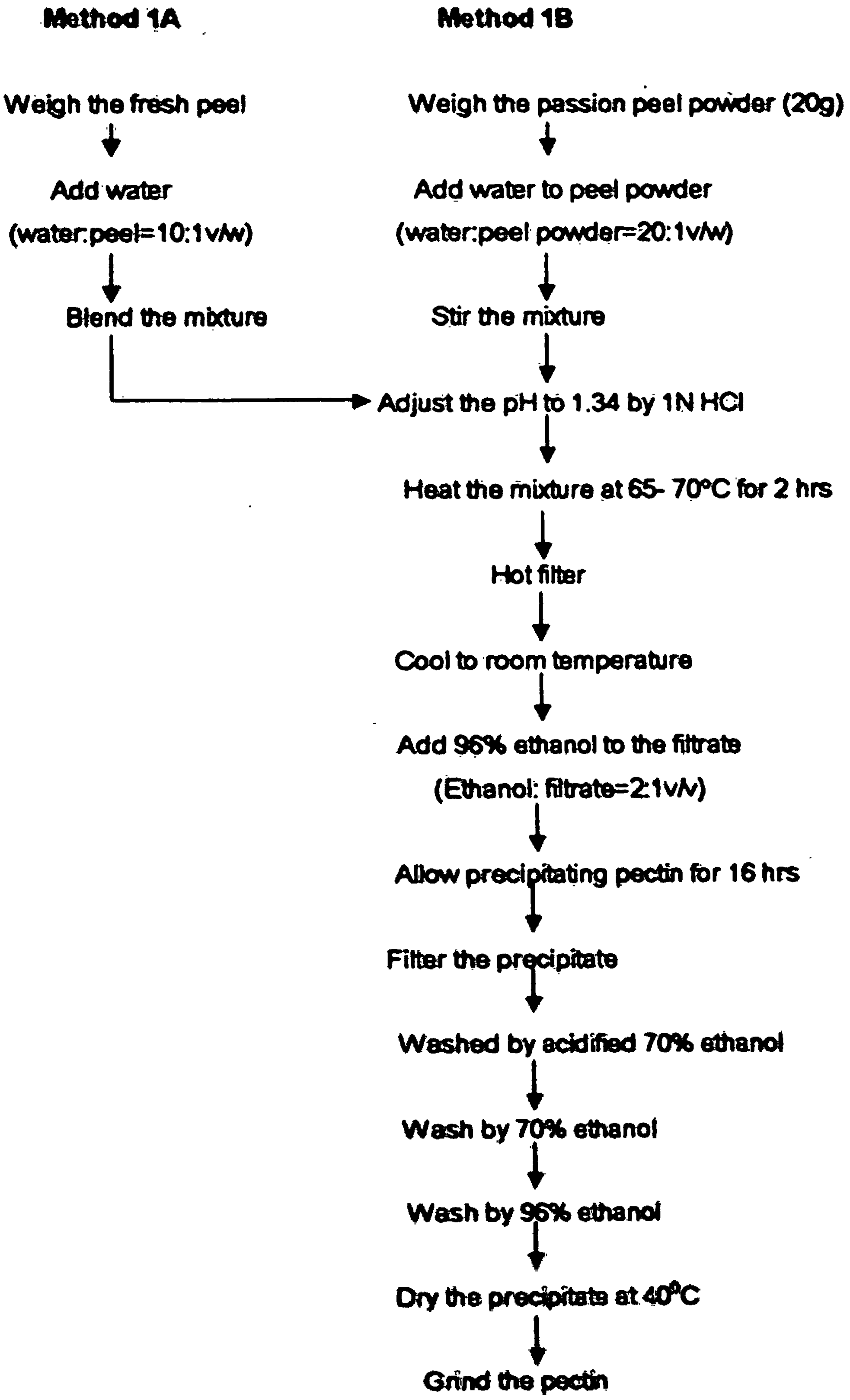


**Figure: 3.1 Extraction of pectin from whole peel**

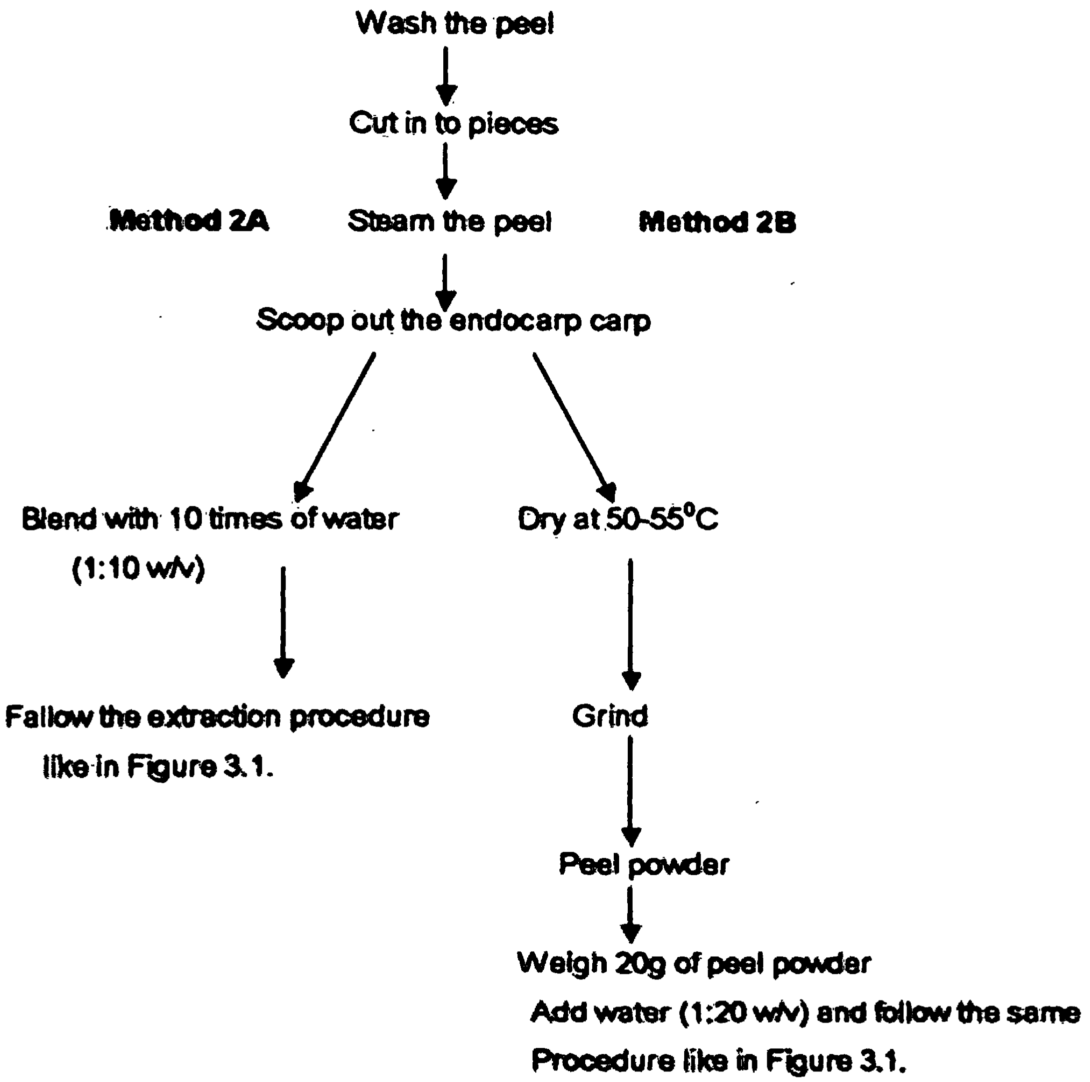


**Figure: 3.2 Method of Pre-processing with removal of outer coat from passion peel (endocarp extraction).**

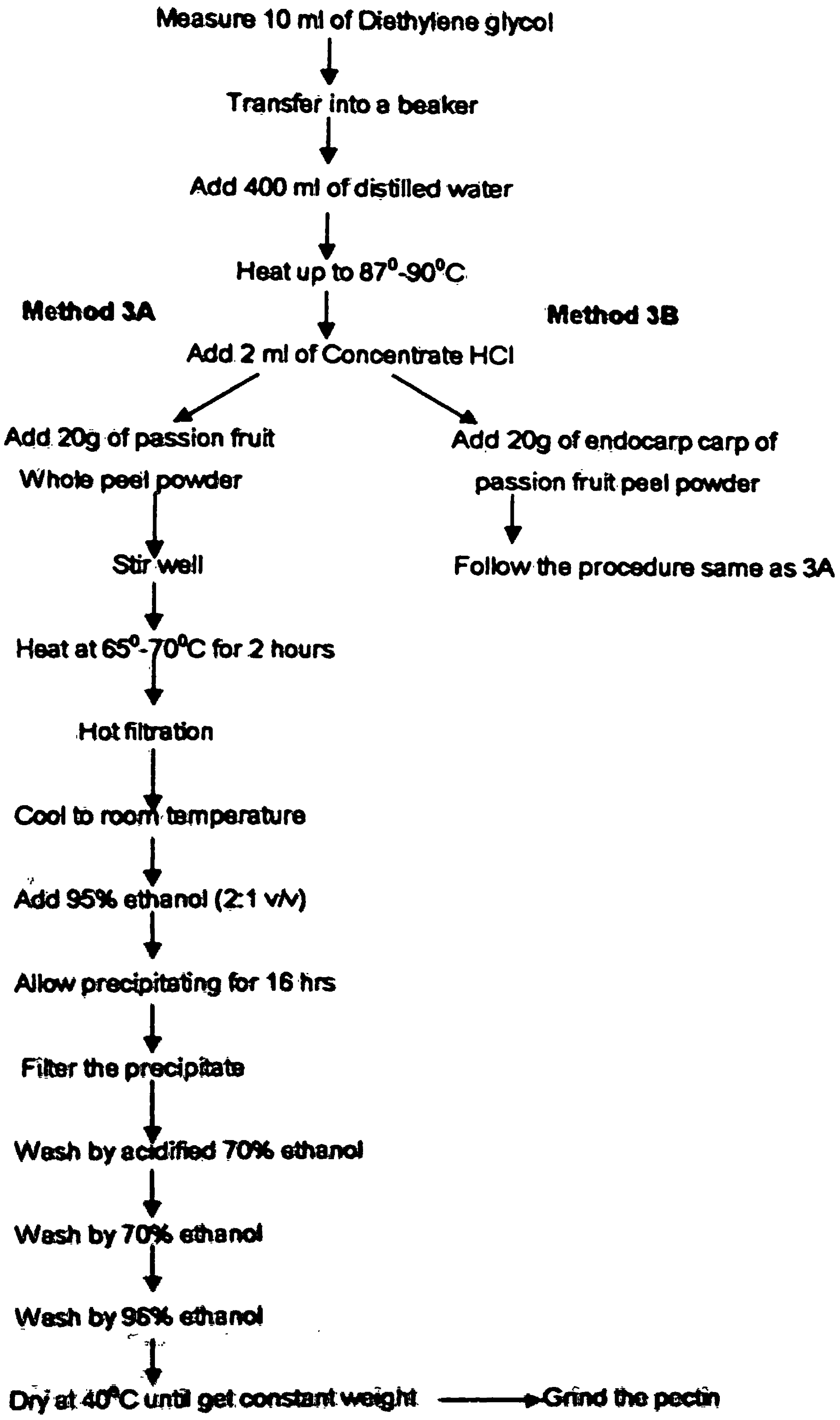
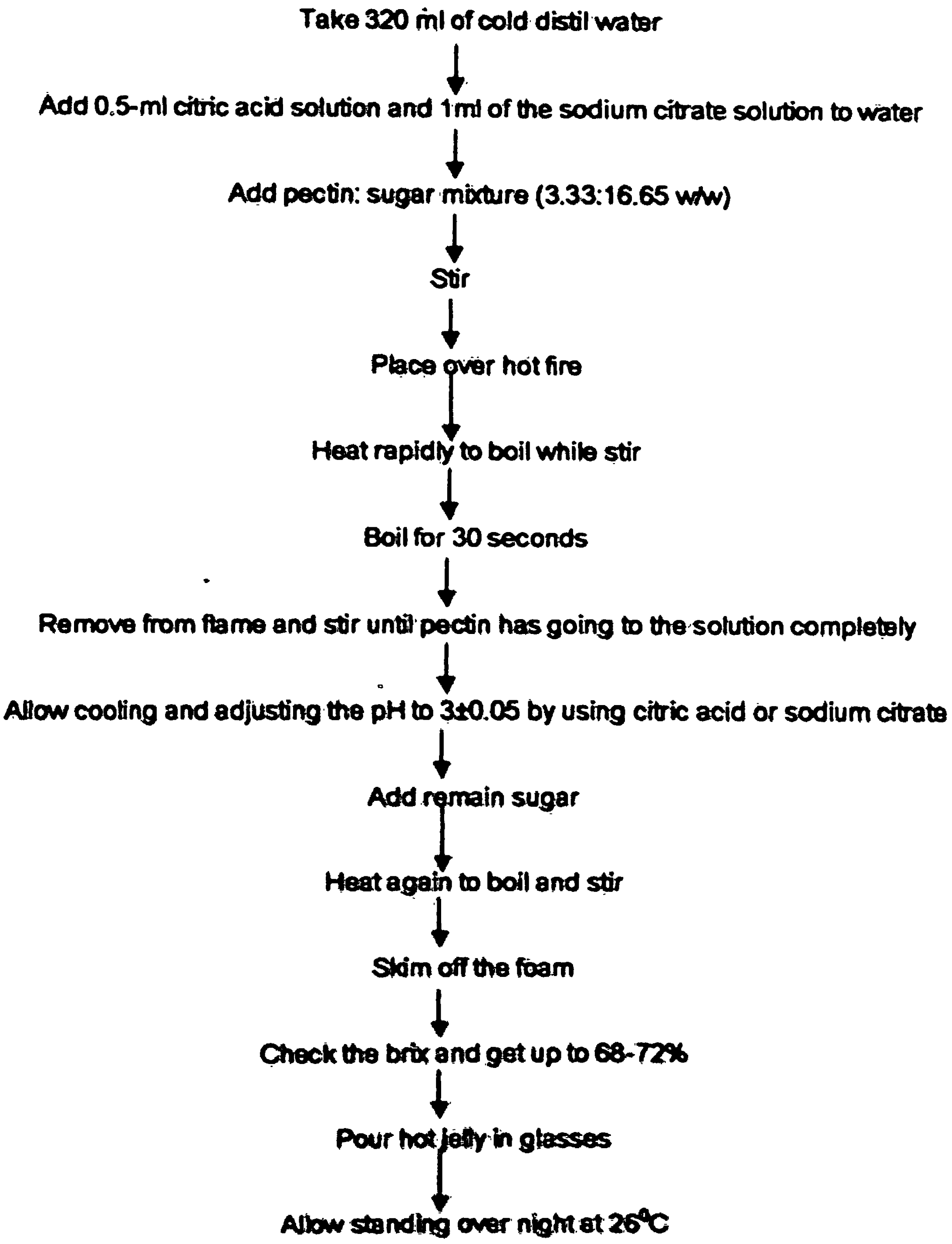


Figure: 3.3 Pectin extraction in the presence of alcohol surfactant (Diethylene glycol)



Take 320 ml of cold distil water

↓

Add 0.5-ml citric acid solution and 1ml of the sodium citrate solution to water

↓

Add pectin: sugar mixture (3.33:16.65 w/w)

↓

Stir

↓

Place over hot fire

↓

Heat rapidly to boil while stir

↓

Boil for 30 seconds

↓

Remove from flame and stir until pectin has going to the solution completely

↓

Allow cooling and adjusting the pH to 3±0.05 by using citric acid or sodium citrate

↓

Add remain sugar

↓

Heat again to boil and stir

↓

Skim off the foam

↓

Check the brix and get up to 68-72%

↓

Pour hot jelly in glasses

↓

Allow standing over night at 26°C

Figure: 3.4 Jelly preparation

### 3.2.2. Chemical analysis of pectin

#### 3.2.2.1. Methoxyl content

Reagents

1. 0.25N and .01N standard Sodium hydroxide
2. 0.25N standard Hydrochloric acid

Procedure:

0.5g of pectin substance was weighed into a 250-ml conical flask, added 25-ml of 0.25N Sodium hydroxide, shacked thoroughly and allowed to stand for 30 min at room temperature in a stoppered flask. Then 25 ml of 0.25N HCl and 6 drops of phenol red were added. After that it was titrated with 0.1N NaOH until the colour of the indicator changed (pH 7.5). The colour change should persist for at least 30 sec (Ranganna, 1986).

Calculation:

$$\text{Methoxyl content \%} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of the sample}}$$

#### 3.2.2.2. Acetyl value

Reagents:

1. 0.05N and 0.1N standard Sodium hydroxide solution
2. Magnesium sulphate - sulphuric acid solution: Mix 100g of magnesium sulphate crystals and 1.5g of $H_2SO_4$ and dilute to 180 ml
3. Phenol red indicator

Procedure:

0.5g of pectin was weighed into a 250 ml conical flask and added 25 ml of 0.1N NaOH. The flask was closed by stopper and stirred the contents until the pectin was dissolved. Then it was set aside for overnight. Then the content was diluted to 50 ml with distilled water, and a 20-ml aliquot was transferred to the distilling flask. A 20-ml portion of magnesium-sulphate-sulphuric acid solution was added.

Steam distillation was carried out and collected about 100 ml of distillate, keeping the volume in the distillation flask low. The acetic acid was titrated with the 0.05N NaOH to a phenol red end point. Blank distillation was carried out using 20 ml distilled water and 20 ml of the magnesium sulphate-sulphuric acid solution and titrated the distillate as describe in the case of the sample. The titre should be less than 0.1 ml of the standard alkali.

Calculation:

$$\% \text{ Acetyl content} = \frac{\text{Normality of NaOH X ml of NaOH X 4.3}}{\text{Wt. of sample in aliquot (g)}}$$

**3.2.2.3.Uronic acid content**

Reagents:

1. 0.025 M Sodium tetraborate. 10 $H_2O$ (Analytical grade) in sulphuric acid. Sp.gr.1.84 (analytical grade).
2. 0.125% Carbazole in absolute ethanol or methanol (analytical grade). Stable for 12 weeks at 4°C in the dark.

Procedure:

5 ml of sulfuric acid reagent was placed in tubes, fixed in a rack and cooled to 4°C; 1ml of standard or 40/50 µl of sample was carefully layered on to the acid. The tubes were closed with teflon stoppers and the rack shaken at first gently, then vigorously with constant cooling. At no time should the temperature of the mixture exceed room temperature can rise to 135°C at the interface. The tubes were then heated for 10 min in a vigorously boiling distilled water bath and cooled to room temperature.

Carbozole reagent (0.2 ml) was then added; the tubes were shaken again, heated in the boiling bath for a further 15 min, and cooled to room temperature. The optical density (OD) is then at 530µm (nm) in a 1cm cell. The OD of the blank against sulfuric acid should be below 0.025.

### 3.2.2.4 Degree of esterification

Reagents:
0.1N NaOH
0.5 N NaOH
0.5 N HCl
Phenolphthalein

Procedure:

Degree of esterification was determined using the titration method of Suchaltz (1965) and Schewiger (1965). 10 ml of 1-% pectin solution was titrated with 0.1N NaOH (titration A). 20 ml 0.5 N NaOH was added to exactly neutralize the NaOH (Titration B), using phenolphthalein as indicator. The degree of esterification was calculated through the following equation.

Calculation:

$$DE = [B/(A+B)] \times 100\%$$

### 2.2.5 Gel grade

Materials:
Sugar
Pectin
Distilled water
Citric acid solution: Dissolve 50g of citric acid (Monohydrate, mol wt 210) in water and dilute to 100ml
Sodium citrate solution: Dissolve 25g of Sodium citrate in distilled water and dilute to 100 ml
Vessel for making jelly: Should be made of Aluminum, stainless steel or glass and of capacity 850ml
Ladle
Laboratory balance
Jelly glassed

**Table: 3.1 Gel grade Table**

Weight of pectin to be used in testing for different grades

| Grade | Weight (g) | Grade | Weight (g) |
|---|---|---|---|
| 10 | 50.00 | 120 | 4.17 |
| 20 | 25.00 | 130 | 3.85 |
| 30 | 16.66 | 140 | 3.57 |
| 40 | 15.50 | 150 | 3.33 |
| 50 | 10.00 | 160 | 3.12 |
| 60 | 8.33 | 170 | 2.94 |
| 70 | 7.14 | 180 | 2.78 |
| 80 | 6.25 | 190 | 2.63 |
| 90 | 5.55 | 200 | 2.50 |
| 100 | 5.00 | 210 | 2.38 |
| 110 | 4.55 | 220 | 2.27 |

Source: (U.S Department of agriculture, 1942)

Procedure:

320 ml of cold distilled water was measured. Weigh 500g of sugar. The assumed grade of pectin was 150, weighed 3.33g of pectin and mixed the pectin with about 5 times its weight of sugar from the 500g sugar. To the water in the kettle, 0.5 ml of the citric acid solution and 1ml of the sodium citrate solution were added. Then the pectin-sugar mixture was added into the water. Stirred to ensure dispersion of pectin. The mixture was placed over a hot fire. Heated rapidly to boiling while stirring constantly to prevent lumping or sticking to the sides of the kettle. Boiled for 30 sec, and it was removed from flame, stirred until pectin has gone into solution completely and allowed to cool down to room temperature and then the pH was adjusted to 3±0.05 by using citric acid or sodium citrate solution. Then the remaining sugar was added and heated the solution again to boiling, stirred continuously and boiled down Removed from the fire occasionally to check the weight, as otherwise, too much water may be evaporated. After that the jelly was removed from the fire and allowed the jelly to cool for 30 sec. Skimmed off the foam and brix value was measured (around 65-75%), and poured into the jelly glasses. The hot jelly was cooled to room temperature first and then allowed to stand at 26°C for above 18 hours.

# CHAPTER- 04

## RESULTS AND DISCUSSION

The peel of the golden yellow passion variety (*Passiflora indica*) was used in this study. The peel is a waste product of the fresh fruit juice industry of Sri Lanka.

### 4.1 Effect of pre-processing on the extraction of pectin from passion peel

Pectin from passion peel was extracted after pre-processing by four different methods, described schematically and presented in Figure 4.1.

The yield of pectin ranged from 14.5 to 29.0 % by weight on dry basis, as presented in Table 4.1.

The results showed that dry powder yielded higher values than when extracted on fresh material.

### 4.2 Chemical characteristics of pectin from passion peel

The methoxyl content, Acetyl value and degree of esterification were carried out as described in 3.2.2, the pectin was used from Method 1 and 2. The results obtained are presented in Table 4.1.

The chemical characteristics show that pectin from passion peel belong to "high methoxy pectins" as the value is above (> 7%). The degree of esterification was 60%. The Acetyl value by Method 1A and 1B showed to be 1.2%. Removal of the skin by steaming (endocarp extraction) decreased the Acetyl value to 0.8%. High Acetyl content of pectin (>3.0%) inhibits gel formation (Mc Cready, 1966).

### 4.3 Extraction of Pectin

The extraction of pectin was carried out at pH 1.34, 65 ° C for 2 hr. It was found essential to maintain the pH and temperature to obtain good quality pectin.

During the extraction, adjusting the pH of media at 1.34 and heating temperature at 65°C should be maintained carefully, because the pH difference and temperature exceeding above 90°C effect the properties of the pectin.

### 4.4 Purity of pectin

The uronic acid content of pectins was determined as the purity of the pectins. The results showed that the uronic acid content was 83.8 % ± 2.1. This value is the mean of duplicate assays carried out five separate sequential extractions with standard deviation. (Hwang, *et.al.* 1992).

### 4.5 Use of surfactants in pectin extraction

Diethylene glycol was used as a surfactant in the acidified extraction procedure of pectins. It was found that the percentage pectin yield increased ranging from 17 to 80. The yield of pectin obtained by Method 1A and 1B and Method 2A and 2B is presented in Table 4.1.

The presence of an acid and the action of temperature help cell wall disruption. This protopectin hydrolysis and stabilisation of pectic substance simultaneously results in the degradation process of pectin macromolecules (Kane, *et. al.* 1983; Thibault and Rombouts, 1985). The addition of organic solvents (mainly different alcohol) stimulates it's penetration in the capillary pore structure of the fruit tissue, act as a surfactant and reduces a surface tension thus contributing to the more subtle pectin extraction. This also improves the extracting wetting ability thus facilitating extractant penetration in the peel tissue. This has a favourable effect on the protopectin hydrolyzation rate (Kirtchev, *et. al.* 1989)

According to the Table 4.2, Method 3A resulted in about 80% increase in the yield while Method 3B showed only a 17% increase. The reason can be that on steaming, the protopectin under go hydrolysis and give the high yield in Method 3B during the pre-processing.

**Table: 4.1 Yield and chemical characteristics of pectin extracted from passion peel**

| Source | Method of pre – processing/ extraction [a] | Yield (dry basis, %± SD) | Methoxyl Content ±SD | Degree of Esterification % ± SD | Acetyl content %± SD |
|---|---|---|---|---|---|
| yellow passion fruit peel | Method 1A | 14.5 ± 1.5 | 9 ± 0.22 | 60 ± 0.42 | 1.2 ± 0.05 |
| | Method 1B | 18 ± 1.5 | | | |
| | Method 2A | 16.2 ± 1.7 | 9 ± 0.12 | 59 ± 0.52 | 0.8 ± 0.07 |
| | Method 2B | 29 ± 1.2 | | | |

[a] see Materials and Methods for extraction procedure.

(Values are presented as a mean of three representatives)

Method 1A: Whole peel, freshly extracted
Method 1B: Whole peel, dried and powdered
Method 2A: Endocarp (after steaming), freshly extracted
Method 2B: Endocarp (after steaming), dried and powdered

**Table: 4.2 Effect of the use of dietheylene glycol as a surfactant on the yield of pectin**

| Source | Yield of pectin % ± SD | |
|---|---|---|
| | Pectin extraction in acidic media * | Pectin extraction in acidic media in the presence of diethylene glycol* |
| Whole peel dried powder (Method 3A) | 18 ± 1.5 | 29 ± 0.8 |
| Endocarp Peel dried powder (Method 3B) | 29 ± 1.2 | 34 ± 0.5 |
| Pumpkin dried powder | 15 ± 2.0 | 20 ± 2.2 |

*See the Material and Methods for extraction procedure.

Values are presented as a mean of three representatives and Standard Deviations.

Method 3A: Whole peel, dried and powdered

Method 3B: Endocarp (after steaming), dried and powdered

**Table: 4.3 Effect of pre-treatment methods on the gelling characteristics of pectin extracted from Passion peel**

| Source | Method of Pre-treatment and extraction [a] | Gel Grade [b] | Setting time (in minutes) | Rupture point (Kp) | Compression (N) |
|---|---|---|---|---|---|
| Citrus | - | 150 | < 30 | 0.7 ± 0.05 | 18 |
| Yellow passion peel | Method 1 A | 140 | Did not set | - | - |
| | Method 1 B | 140 | Did not set | - | - |
| | Method 2 A | 140 | not determined | - | - |
| | Method 2 B | 140 | < 30 | 0.6 ± 0.05 | 13 |
| | Method 3A | 140 | < 30 | 0.7 ± 0.02 | 18 |
| | Method 3B | 140 | < 30 | 1.1 ± 0.04 | 30.1 |

[a] see materials and method for extraction procedure

[b] a capacity of pectin to gel with sugar

Values are presented as a mean of five representatives

Method 1A: Whole peel, freshly extracted

Method 1B: Whole peel, dried and powdered

Method 2A: Endocarp (after steaming), freshly extracted

Method 2B: Endocarp (after steaming), dried and powdered

Method 3A: Whole peel, dried, powdered and extracted in the presence of alcohol surfactant

Method 3B: Endocarp (after steaming), dried, powdered and extracted in the presence of alcohol surfactant

**Table: 4.4 A comparison of the chemical and functional characteristics of pectin extracted from different fruit sources**

| | Citrus (Commercial) | Passion Peel (Method 2) | Pumpkin* |
|---|---|---|---|
| Methoxyl Content % | 8.6 | 9.0 | 8.2 |
| Degree of Esterification % | 78.21 | 59.0 | 66.7 |
| Acetyl Value % | 0.32 | 0.8 | 0.99 |
| Uronic Acid % | 77.42 | 83.8 | 50.65 |
| Gel grade | 150 | 140 | 100 |
| Rupture Point (Kp) | 0.7 | 0.6 | 0.3 |
| Compression (N) | 18.0 | 13.0 | 1.5 |

* Pectin from pumpkin was extracted in the laboratory.

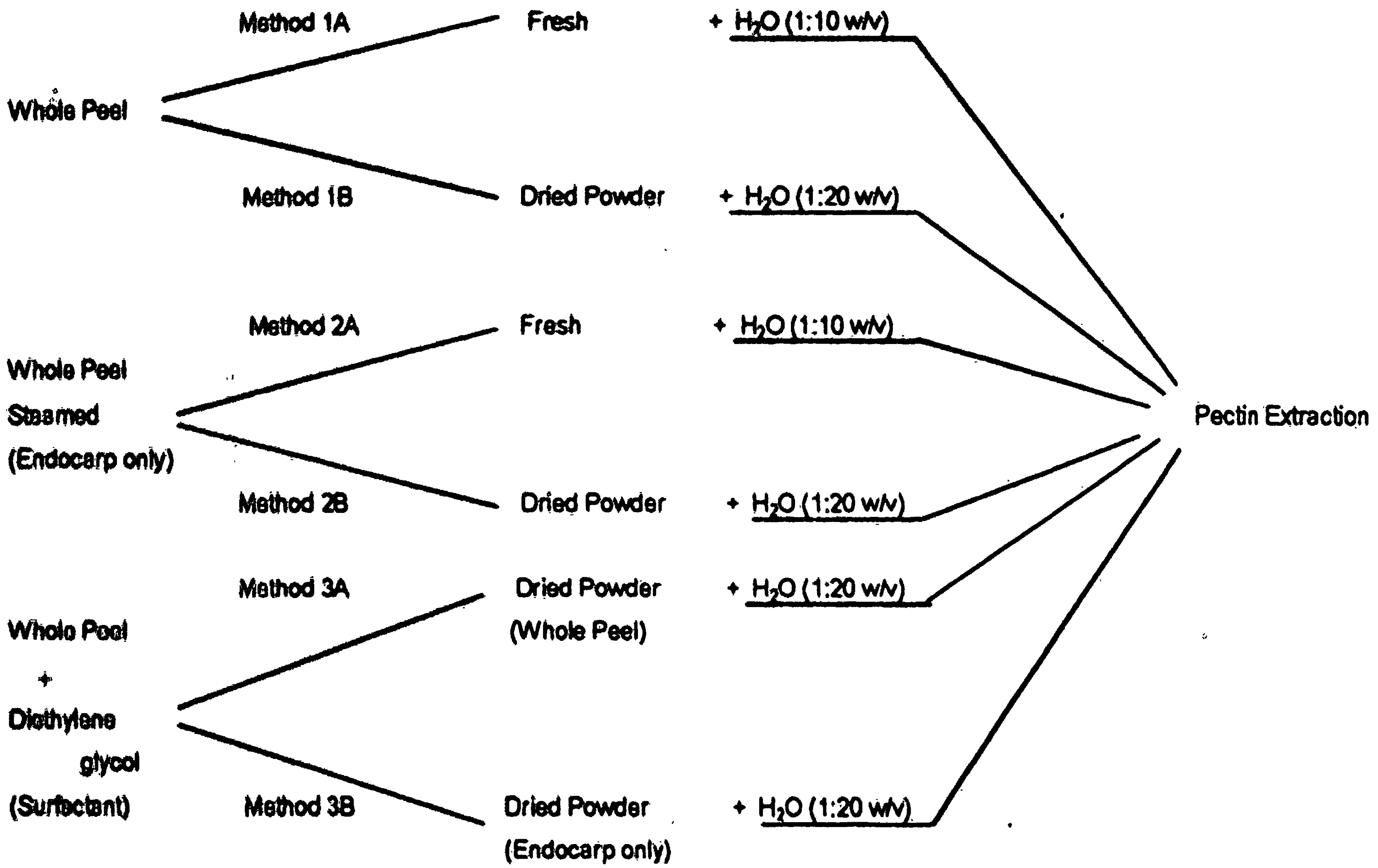


**Figure: 4.1 Schematic diagram on pre-processing method of Passion fruit peel prior to pectin extraction**

### 4.6 Gelling characteristics of pectin

Gelling characteristic of pectin is determined by the chemical characteristics of pectin. The passion peel pectin is a high methyl (HM) and rapid setting type pectin, because it has methoxyl content above 7% and degree of esterification (DE) above 50%. Though the pectin is HM, when pectin was extracted from the whole peel its properties had changed, which gave a spreading sticky texture jelly. The reason can be attributed to the high acetyl content. High acetyl content of pectin decreases the gelling capacity (Mc Ready, 1966) however the use of diethyleneglycol as surfactant had improved the gelling properties.

Normally high methoxyl pectins from jelly at 150 grade, but extraction by Method 1A and method 1B did not form jelly at this grade and therefore the experiments were repeated at gel grade 140. (Grade is defined as Table: 3.1, indicating the quantity of sugar which, when used with a unit amount of pectin, will produce as standard gel.) In these preparations commercially available citrus pectin was used as the standard gel.

Jelly is formed with high sugar content (65-75%) and at pH 3. In addition to the strength of the gel setting characteristics, the degree of methylation of the carboxyl acid group in the molecule are also of vital importance. Gelling characters is the most important factor to determine the industrial application of pectin. Results show that pectin from passion gel can form gels similar to that formed with citrus gels. Method 3A pectin gives similar strength of jelly as citrus pectin.

An interesting observation was that passion gels were not very transparent like citrus jelly, due to the colour of the passion pectin. This colour can be removed by using activated carbon (Girdhairi, *et. al.* 1986)

Method 2A pectin jelly also has acceptable quality to industrial application, but was more rigid than seen for pectin from Method 3B. (Compression Force (N) = 30>18). It is very high and was a very rapidly setting jelly. Surfactant application to the extracting increased the strength of jelly as shown in Table: 4 3.
A comparison of pectins from two different sources (Table 4 4), passion peel and pumpkin showed that passion peel gels had all characteristics similar to citrus pectins.

# CHAPTER- 05

## CONCLUSIONS

The following conclusions can be drawn from this study

5.1 Passion peel is good source for pectin, with a yield of approximately 14-29.0% and a purity of 83.8%. It can be classified as a high methyl type pectin based on its methyl content (> 7%) and degree of esterification (>50%).

5.2 The application of a pre-treatment method (Method 2A and 2B) such as steaming improved the gel forming characteristics of the pectins. This may be attributed to the lowering of the % Acetyl Value of the pectin.

5.3 The use of a surfactant such as diethylene glycol increased the yield of pectin significantly by 50-85% and gel strength of the pectin.

5.4 Gel-forming properties of passion peel pectin was found to be similar to that citrus pectin.

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