CHAPTER -1: INTRODUCTION

During the peak season huge amount of citrus fruits such as orange, lemon, lime, sweet lemon, mandarin, grape fruit etc are grown in Bangladesh due to favourable climatic conditions. Among all fruits, lemon products are an important citrus crop in Bangladesh which has annual production of 20,000 tons (BBS, 2010). Usually fruits are processed into juice, beverage, squash and syrups. During processing, the peel contributes almost 5-20% and seed contributes almost 5-10% of the total fruit. Therefore, huge amount of by-products are generated during processing that are responsible for pollution of the environment. One of the major challenges in the food industry throughout the world is how to make full utilization of the waste materials.

Sweet lemon (*Citrus sinensis*) is a citrus fruit, most commonly grown tree fruit in the world known as 'Malta'. This tree fruit is also one of the important citrus fruits grown in Bangladesh. Citrus fruits are at the top not only in total production, but also in economic value. Citrus fruit consists of two parts namely the peels (rind skin) and pulp and these two parts are easily separated from each other. The pulp serving as the edible parts of the fruit while the peels contain many useful components which can be extracted and utilized in different products. One of these components is pectin. Hence, citrus peel has become one of the most important sources of commercial pectin.

Pectin is a heteropolysaccharide found in the primary cell wall of many plants. It is a white to light brown powder, mainly extracted from citrus fruits, and is used as a gelling agent particularly in jams and jellies, and also used in medicines, sweets, stabilizer in fruit juices and milk drinks.

Pectin is one of the most valuable products which can be obtained from various sources. Commercial pectins are primarily extracted from citrus peel such as lime peel, guava extract, apple pomace (Chakraborty and Ray, 2011), and orange (Braddock 2004). Other source of pectin includes cocoa husk (Mollea et al., 2008), sunflower heads (Matora et al., 1995), beet and potato pulp (Turquois et al., 1999), soy hull (Kalapathy & Proctor, 2001) and duckweed (Golovchenko et al., 2002). Pectin extraction is usually accomplished with water, inorganic acids such as

hydrochloric acid, nitric acids, sulphuric acids and phosphoric acid. Extraction of pectin also depends on various factors such as extraction time, temperature, P^H and types of extraction solvent (Koubala et al., 2008b). Extraction time 20 to 60 minutes, extraction temperature 60 to 100˚C and P^H1.4 to 2.6 are used for extraction of pectin from various sources (Chakarborty and Ray, 2011).

An extraction process is the most important operation to obtain pectin from plant tissue. Pectin extraction is a multiple-stage physiochemical process in which hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, P^H and time.

The main use of pectin is as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in gelling sugar (also known as "jam sugar") where it is diluted to the right concentration with sugar and some citric acid to adjust the P^H. In some countries, pectin is also available as a solution or an extract, or as a blended powder for home jam making.

The present work is dedicated for the development of the part of the process technology needed for the extraction of value added products i.e., pectin from sweet lemon peel powder, which is the waste of citrus juice processing industry. The present work revealed that the lemon peels are good source of pectin and does have the potential to become important raw material for food processing industries. The citrus processing industry can get a complete makeover if due importance is given for separation of useful ingredient from lemon peel. Researchers and Scientists have been working on the separation of pectin from lemon peel and reporting their findings in journals. Since pectin industry doesn’t exist in Bangladesh, the utilization of citrus peel as a source of pectin might be economically sound and reduces its importation from other countries.

In recent years the production of fruits and vegetables has been increased. Therefore, large amount of fruits and vegetables could be used as value added products as well as good source of pectin. With regard to the present condition a research was conducted

to find out the various extraction conditions such as extraction solvent, extraction time and extraction temperature for pectin extraction from sweet lemon.

Hence the present study is undertaken to establish a feasible and effective optimum extraction condition of pectin from the waste citrus peels as a waste utilization from nearby local fruit juice market and it's utilization in the production of jelly.

The major objectives of this study as following:

i. To extract pectin from Malta peel by various extraction conditions.
ii. To determine the physiochemical parameters and utilization of extracted pectin in the production of jelly.
iii. To evaluate the sensory quality and determination of microbial analysis of the prepared jelly.

CHAPTER -2: REVIEW OF LITERATURE

2.1 Pectin

Pectin exists in varying amounts in fruit cell walls and has important nutritional and technological properties, mainly because of its ability to form gels (Westerlund et al., 1991). The pectin is used in manufacture of jams, jellies, marmalades etc (Thakur et al., 1997). It is also useful as a thickening agent for sauces, ketchups, flavoured syrups and as a texturising agent in fruit-flavoured milk deserts. Besides, it has numerous applications in pharmaceutical preparations, pastes, cosmetics etc, but it had also been shown to have potential as a serum cholesterol-lowering and hemostatic agent, a demulcent, and a compound preventing spontaneous cancer metastasis (Jackson et al., 2007).

Pectins have several biological and physiological functions in human nutrition and health. Pectin has become highly valued as a soluble dietary fiber (Yamada, 1996). As dietary fibers pectic polysaccharides are able to regulate the lipid metabolism (Groudeva et al., 1997) to reduce the absorption of glucose in the serum of diabetics (Schwartz et al., 1988) and to intensify the detoxification from heavy metals (Stantschev et al., 1979). Pectic polysaccharides can prevent the adherence of pathogens on the intestinal mucosa and are fermented by probiotic bacteria into short-chain fatty acids, which inhibit necrotic processes in the colon (Wang and Friedman, 1998).Pectin is regarded as a safe ingredient for use in food and non-food formulation. Low methoxyl pectin is increasingly being used as a fat replacer in dietetic foods and has received special attention by the food industry (Shi et al., 1996).

2.2 Extraction of pectin by different methods

The most common method for extraction of pectin includes Direct boiling and Microwave heating (Yeoh et al., 2008). Direct boiling is a conventional method of pectin extraction, which takes approximately two hours to obtain a good yield of pectin. Due to a relatively long period of direct heating, the extracted pectin undergoes thermal degradation (Yujaroen et al., 2008). Microwave heating extraction, on the other hand, takes no more than fifteen minutes to extract a satisfactory amount of pectin. Pectin extraction depended on the types of extraction solvents used and the

use of extra chelating agents such as EDTA and CDTA which helped in releasing pectin from cell wall (Marshal and Chou, 2007).

2.2.1 Water based extraction

Extraction of pectin with the hot water was the simplest and oldest method for removing the pectic substances. The most commonly used acidifying materials were mineral acids including sulfuric, hydrochloric and phosphoric acids. Many organic acids and their salts such as oxalic acid, ammonium oxalate, tartaric acid, polyphosphates, and many others had also been used (Srivastava and Malviya, 2011).
Joseph and Huang (1973) worked on a novel method based on IER (Ion exchange resin) and mineral acid. Adjustment of proper P^H had given increased yield of pectin with quality maintained at the highest levels and maximum pectin extracted at P^H of 1.6 for a preferred sulfonated resin. Commercially, pectin was extracted by treating the raw material with hot dilute mineral acid at P^H about 2.0 (Joye and Luzio, 2000).

Huong and Luyen (1989) mixed 60g of orange peel, 1800mL of water and 18g of IER (Ion Exchange Resin) and heated the mixture to 85°C with constant stirring for 1 hour. The solid materials were filtered and pectin yield was found to be 20.5%.
Pectins were industrially extracted from citrus peels and apple pomace by hot acidified water. Extraction conditions of P^H1.5 to 3.0 and temperatures of 60-100°C for 0.5 to 6 hours were varied to get a material that had the desired gelling capacity and degree of methylation. But the separation of the viscous material from the swollen and partially disintegrated plant material remained a problem. Grinding and washing with ethanol were used but this could lead to co-precipitation with intracellular proteins, starches and nucleic acids (Fertonani et al., 2009).
Hoshino et al. (2009) used sub critical water for extracting pectin from the residue of citrus juices in semi continuous flow reactor at temperature range of 110-160°C and pressure range of 4-30mpa. They found that yield of pectin was more at the temperature range from 120 to 140°C and pressure range from 4 to 30mpa as compared to temperature less than 120°C and same pressure. They also indicated that molecular weight and degree of esterification of pectin was affected by changing temperature. Pectin extracted using acidified water with P^H up to 2.0 and temperature 70°C. The assembly was run for two hours duration and peptic substances were precipitated using isopropyl alcohol (Srivastava et al., 2010).

2.2.2 Microwave heating extraction

Zhongdong et al. (2006) researched the process of pectin extraction from orange skin by different methods, i.e. traditional method and microwave heating method. They found that microwave radiation had the stronger destructive effect on the structure of orange skin organization and the swelling effect of the microwave even forced the cells to split. Yoeh et al. (2008) compared the different techniques for water based extraction of pectin from orange peel and found that with microwave extraction, the greatest amount of material per unit time (% min) was obtained after 5m, which was the same amount as that extracted using soxhlet extraction for 3 hours.

Fishman et al. (2009) studied the overall structure of microwave-assisted alkaline soluble polysaccharides isolated from fresh sugar beet pulp to minimize the disassembly and possibly the degradation of these polysaccharides during extraction. Prior to alkaline soluble polysaccharides, pectin was extracted by microwave-assisted extraction using HCl at P^H1.0 in 3m and 30psi pressure irradiating with 1200W of microwave power at a frequency of 2450MHz.

Microwave extraction had been carried out included 10% ethanol, 0.05M EDTA, 1M sodium hydroxide which was used to maintain P^H up to 12 or less. Compared with water based extraction, microwave heating extraction reduced the extraction period considerably. A 15 minute microwave heating period was enough to extract almost the same amount of pectin that obtained from water based extraction with a three hour extraction period. Microwave extraction also gave a higher rate and amount of extraction than the simple water based methods (Fishman et al., 2000). According to Srivastava et al. (2010) solvent used in microwave extraction include 10% ethanol, 0.05M EDTA, 1M NaOH which was used to maintain P^H up to 2.0 or less. Microwave heating extraction reduces the extraction period as compared with water based extraction. Calce et al. (2012) developed a solvent-free synthetic process in order to modify pectin via acylation of alcoholic functions of the polysaccharide by using several fatty acid anhydrides and replaced traditional heating with microwave heating.

2.2.3 Extraction by pectic enzymes

Pectinases were group of enzymes that attack pectin and depolymerise it by hydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 1998). Agricultural and food processing wastes such as wheat bran, cassava,

sugar beet pulp, citrus waste, corn cob, banana waste, saw dust and fruit pomace (apple pomace) are the most commonly used substrates pectinase production (Pandey et al., 2002).

Sakai and Okushima (1980) developed a method for production of pectin from citrus peel using microorganism, *Trichosporom penicillatum* which produced a protopectin solubilizing enzyme. Citrus peel was suspended in water (1:2w/v), the organism was added and fermentation proceeded over 15 to 20h at 30°C. During the fermentation, the pectin in peel was extracted almost completely without macerating the peel and 20 to 25g pectin was obtained per kg of peel. The pectin obtained contained neutral sugar at high levels which was determined to have a molecular weight suitable for practical applications.

An optimized procedure was reported for extraction of pectin from pumpkin pulp, using an enzyme preparation from *Aspergillus awamori*. In contrast to pumpkin pectin obtained by digestion with cell-free culture medium from *Bacillus polymyxa*, this pectin formed gels with 60% sucrose at P^{H}3.0, although the yield was somewhat lower, 14% in comparison with 22, found cell free culture medium of *Bacillus polymyxa.* The main action of the enzyme complex from Awamori was to degrade cellulose and other insoluble constituents of the plant tissue, but it also had some pectinesterase activity, which could allow degree of esterification (DE) to be manipulated by varying digestion time (Ptichkina et al., 2007).

Pectin from Yuza (Citrus junos) pomace was extracted by using combined physical and enzymatic (CPE) treatment and their characteristics were compared with those of chemically extracted pectin. Their physico-chemical and thermo-mechanical properties were also investigated in a wheat floured water system. The CPE extraction produced pectin with 55% of galacturonic acid and the extraction yield was 7.3%, whereas pectin extracted with chemicals had galacturonic acid content 72% and yield 8% (Lim et al., 2012).

2.3 Purification and characterization of pectin

The extracted Krueo Ma Noy (*Cissampelos pareira*) pectin was low methoxyl pectin which consisted mainly of uronic (galacturonic) acid (70–75%) and small amounts of neutral sugars. Gelation was observed when Krueo Ma Noy pectin (both crude extract and dialyzed fractions) concentration exceeded 0.5% (w/v); gel strength of the dialyzed fraction was much higher than that of the crude extract (Singthong et al., 2005).Total galacturonic acid released after acid hydrolysis of a pectin sample of

papaya fruits was determined colorimetrically using a modified m-hydroxydiphenyl sulfuric acid method and found to be 72.43%. It was reported that galacturonic acid content is maximum at green stage of papaya fruits (Boonrod et al., 2006).

Sharma et al. (2006) used sodium dodecyl sulphate (SDS) for enzymatic degradation of the pectin. Sodium deoxycholate (SDC) was used to remove pigments and lipids and 90% dimethyl sulphoxide (DMSO) was used to remove the bulk of the starch. They stated the advantage of alcohol treatment that the resulting preparation was suitable for further modification to high methoxylated (HM) pectins using acid treatment or to low methoxylated pectins (LM) by treatment with ammonia, whereas the disadvantage of alcoholic treatment could be the possibility of reinforcing hydrogen bonding between cell wall constituents that effecting the extraction of the pectins.

Apple pomace which is the main waste of fruit juice industry was utilized to extract pectins in an environmentally friendly way, which was then compared with chemically extracted pectins. The water-based extraction with combined physical and enzymatic treatments produced pectins with 693.2mg/g galacturonic acid and 4.6% yield, which were less than those of chemically-extracted pectins. Chemically-extracted pectins exhibited lower degree of esterification (58%) than the pectin samples obtained by physical/enzymatic treatments (69%), which were also confirmed by FT-IR analysis (Min et al., 2011).

2.4 Effects of acid concentration, temperature and time on the process and the product

Rouse and Crandall (1976) extracted pectin from grapefruit, orange and lemon peels with nitric acid. Pectin extracted from peel was evaluated for yield, jelly grade and jelly units. Maximum yields of pectin calculated to 150 grades, obtained from lemon, orange and grapefruit were 11.0, 8.15 and 6.35%, respectively, while the highest jelly grades were 254, 225 and 263, respectively. The higher jelly units were found for lemon peel (16.5), followed by orange (12.2), and grape-fruit (9.5).

Muralikrishna and Tharanathan (1994) extracted 1.43-5.37% pectin by soaking pulse husk in solvent acidified with HCl and EDTA solution at 70°C, whereas extracted 9-10% good quality pectin from potato wastes (Chen et al., 1999). Ehrlich (1997) suggested that P^H4.5 and 80°C was the suitable condition for the extraction of water-

soluble pectin from *C. maxima*. The P^H 4.5 was the natural acidity produced by acid composition in the peel.

Pagan et al. (1999) studied the extraction of pectin from fresh peach pomace at different conditions of temperature (40°C, 60°C and 80°C), acidity (P^H1.20-2.53) and time (10-80m). The highest yield of pectin was found at the highest temperature and at the lowest assayed P^H. They suggested that industrial pectin of good quality could be prepared from peach pomace. A central composite design was employed by Eloisa et al. (2008) to optimize the extraction of pectin with citric acid. The independent variables were citric acid concentration (0.086–2.91%) and extraction time (17–102m). Satisfactory condition of 0.086% citric acid for 60m was established for extraction of high-ester yellow passion fruit pectin. Heloisa et al. (2009) obtained 19.01g/100g low methoxyl pectin (DE=50.78%) from apple pomace with 126mM HNO3 at 10m of extraction time.

Hoshino et al. (2009) found that the yield of pectin was more at the temperature range from 120-140°C and pressure range from 4-30MPa and they lacked the amount of impurities. On the other hand, the pectin extracted at 120°C and 140°C had the same molecular weight of commercial pectin whereas the molecular weight of pectin was lower when it is extracted at higher temperature (160˚C). They concluded that pectin of different molecular weight could be recovered by changing the extraction temperature.

Pectin was extracted from passion fruit peel using three different kind of acids (citric, hydrochloric or nitric) at different temperatures (40–90°C), P^H(1.2–2.6) and extraction times (10–90m), with and without skins. The processing parameters such as temperature, P^H and extraction time had highly significant effects on the pectin yield. Pectin yields varied from 10% to 70%. The optimal conditions for maximization of pectin yield were the use of citric acid at 80°C and P^H1.0 with an extraction time of 10m considering model extrapolation (Kliemann et al., 2009).

Monsoor and Proctor (2002) studied the effect of various ratios of soy hull to extraction solvent (1:10, 1:15, 1:20, and 1:25) on the yield and purity of soy hull pectin. The soy hull pectin extracts contained 63.07 to 68.72% galacturonic acid at various hull/solvent ratios. The pectin yield increased from 7.68 to 13.73% as the hull/solvent ratio increased from 1:10 to 1:15. The changes in pectin yield for higher hull/solvent ratio were insignificant (16.31–13.28% for 1:20–1:25 ratio, respectively). The hull/extraction solvent ratio did not significantly affect in pectin content and

degree of esterification. Pectin extraction yield from different sources may vary depending on processing parameters (P^H, time, temperature) and sample features. Yield of pectin extracted from chickpea husk was 8% on a dry matter basis, which is lower than those reported in major sources of pectic substances like apple fruit (16%) (Orona et al., 2010).

Drazya and Jechna (1996) studied the quantity of pectin preparation in relation to extraction of P^H. Result shows that there was an increase in the acidity extraction. They reported that the degree of etherification polymerization and gelatin capacity decreases steadity, yield in terms of gelatine capacity showed no clear trends.

2.5 Jelly

Fruit jelly is a semi-solid product prepared by boiling a clear strained solution of fruit juice or sound quality fruits, free from pulp, after addition of required amount of sugar, citric acid and pectin and concentrating to such a consistency that gelatinization takes place on cooling Stavrov et al. (1997). Minimum 65% of total soluble solids and minimum 45% of fruit portion should be recommended for fruit jelly (Dhawan, 1998).

2.6 Health benefits of fruit jellies

A lot of people love having gourmet jams, jellies because they taste so incredibly awesome and there are a lot of positive, body friendly, healthy health befits to eating these delicious “spreadable,” edible fruits. Fruit are normally low in fat, sodium, and calories and have absolutely no cholesterol and are an important source of many vital nutrients, including dietary fibre, vitamin C, folic acid and potassium. As it turns out, according to both medical doctors and naturalistic nutritionists, as well as university researchers and numerous medical studies done all across the globe. Fruit jellies provide our bodies with beneficial quick boosts of energy. Jelly may help to i) stabilize, attain and maintain optimal weight control ii) provide energy and endurance during stress and exercise iii) reduce risk of stroke, heart attack and all other potential cardiovascular diseases iv) reduce the chance of and developing various cancers and possibly help to cure current cancers, particularly mouth, stomach, and colon-rectum cancer v) satiate hunger, helping us to eat less and be satisfied, without craving vi) reduce the chance of developing type-2 diabetes vii) slow down the aging process

viii) reduce constipation and diverticulitis ix) promote healthy growth and repair of all body tissues x) keep teeth and gums healthy.

2.7 Industrial demand for fruit jellies

The jelly market continues to grow at a steady pace, especially at a time when healthy eating is the focus of many consumers. The global jelly market is expected to reach US $2 billion with a CAGR growth of 3.9% during the period 2018-2023.The jellies are indulgent as well as nutritious for the body. Increased use of jellies in fast foods is a major driver for this market. Also, the rising demand for organic food products and the health benefits should be this offer has supported the growth of the market. For the manufacturing of jellies, varieties of materials like fruits and nuts are required. The rising cost of these ingredients is a major challenge for all the vendors. Increasing prices of jellies and jams have helped J.M. Smucker balance out high input costs. To increase its revenue, B&G Foods acquired the Green Giant brand in November 2015, marking the company's entry into the frozen food segment. The global jelly market is segmented by the product type into - fruit jelly, vegetable jelly, herbs and flower jelly, liqueur jelly, and others. Fruit jelly, containing strawberry, was the most consumed jelly flavor, globally, in the year 2016. The report discusses the market revenue of the distribution channels such as specialty stores, independent stores, online sales, convenience stores, supermarkets, hypermarkets, etc. The jelly market has been geographically segmented into North America, Europe, South America, Africa and Asia-Pacific. The market is currently dominated by the European region, especially the Western European countries. France is the major consumer in Europe, followed by Germany and the UK; In which it is a tradition to consume jams and preserves with breakfast. Regarding the growth prospects, developing countries of Asia-Pacific are projected to have the highest growth during the forecast period (Carlson et al., 2012).

CHAPTER -3: MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Applied Chemistry and Chemical Engineering, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University, Khulshi-4225, Chattogram, during the period of 'January-June' 2019.

3.1 Materials

The experimental materials such as malta, pineapple and citric acid were collected from the local Market of Chattogram. The malta were carefully chosen in order to obtained the optimum maturity because its pectin contain depends on maturity. Sugar and citric acid were purchased from scientific and surgical store. Other relevant materials required for the experiment were received from the laboratory stocks.

3.2 Methods

3.2.1 Sample preparation

The malta were peeled and washed in order to remove dirt, dust and the residues of the pesticide spray. They were cut into small pieces, then blanching with boiling water for 5 minutes to inactivate the enzyme. Then filtered by hands through two cheese cloths or muslin cloths, after which the insoluble materials (pieces) were treated in warm absolute ethanol for 30 minutes to remove oil from peel and then washed. After that it was pressed under hand pressure to remove excess water. Thus the alcohol-insoluble solids (AIS) was obtained from malta peel pieces. After that it was dried at 60^{o}C in tray drier until the weight comes constant, then grinded and stored in tightly closed container i.e aluminium coated polyethylene bag at room temperature until use.

3.2.2 Extraction of pectin

The extraction procedure was based on method given by Kratchanova et al. (2012) considering several variables. Total 5g of the peel powder was weighed and put into 250mL conical flask and 150mL distilled water was added. Citric acid and nitric acid were added for maintaining different P^{H} medium as reagents. Extraction was done by

hot water bath procedure. Thereafter, the mixture was heated for each different P^H medium of extraction while stirred at 60, 70 and 80°C for each different time of 30 and 60 minutes. The hot acid extract was filtered through muslin cloth. For each acid, three different P^H medium of extraction at three different ranges of time and temperature, extraction was carried out and collected the extract separately for further experiments. The filtrate was allowed to cool at room temperature.

3.2.3 Purification and Centrifugation

Pectin containing aqueous extract was coagulated by using an equal volume (1:1) of 99.1% ethanol at room temperature and was allowed for 3 hour. The precipitate (ethanol-insoluble fraction) was recovered through centrifugation and filtration, was washed with 55% and then with 75% ethanol.

3.2.4 Preparation of pineapple jelly

For production of pineapple jellies the used ingredients were fresh pineapples and sucrose. At the beginning, total 500g of pineapples were macerated at room temperature 20°C for approximately 5 minutes and the mixture was heated to boiling for 10 minutes. Subsequently, the mixture was filtered through a strainer and the sugar added at a ratio of 1:2 (1g of sugar per 2ml of liquid). Afterwards, the solution was again taken for boiling until a Total Soluble Solids (TSS) value between 65-70°Brix (Lago et al., 2006; Lago-Vanzela et al., 2011), then measured on an Abbe refractometer (Optic Ivymen System, Madrid, Spain). Before cooling the mixture was poured into glass jars with 250g capacity and closed with metal caps. Then the jars were placed in a hot water bath (100ºC) for 15 minutes (Granada et al., 2005) and allowed to cool at room temperature (20°C).

3.3 Analysis of physiochemical properties of extracted pectin

3.3.1 Percentage yield of pectin

The percentage yield of pectin was calculated of all the pectin samples extracted under different treatment conditions.
Yield of pectin was calculated by the following formula:

$$\text{Percentage yield of pectin} = \frac{\text{Pectin obtained (g)}}{\text{Initial amount of peel or pomace powder (g)}} \times 100$$

Here, Total amount of peel or pomace powder (g) =5g.

3.3.2 Moisture content

Total 1.0g of sample was weighed in desiccators and dried in an oven for 4 hours at 100°C. Then it was allowed to cool over silica gel. Percent moisture was observed by the Fischer method. Percent moister content was calculated by using following formula:

$$\% \text{ Moisture content} = \frac{IW - FW}{IW} \times 100$$

Where,

IW = Initial weight of pectin

FW= Final weight

3.3.3 Ash content

Ash content of pectin was determined by the method of Ranganna (1994). Total 1.2g pectic substance (sample) was ignited slowly, and then heated for 3-4 hours at 600ºC. Then cooled the crucible at room temperature in a desiccator and weighted properly. The process will be weighted until constant weight comes and the final weight will be noticed.

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where,

W1 = the weight of dried empty crucible

W2 = the weight of dried empty crucible with sample

W3 = the weight of the crucible with ash

3.3.4 Equivalent weight:

Equivalent weight is used for calculating the anhydrouronic acid content and the degree of esterification. It is determined by titration with sodium hydroxide at P^H7.5 by using either phenol red or Hinton's red indicator. Equivalent weight was determined by Ranganna's method (1994). Total 0.5g sample was taken in a 250mL conical flask and 5mL ethanol was added. Then 1g of sodium chloride to sharpen the end point and 100mL of distilled water were added. Finally 6 drops of phenol red or Hinton's indicator was added and titrated against 0.1N NaOH. Titration point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content.

Equivalent weight was calculated using the following formula:

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

3.3.5 Methoxyl content (MeO)

The methoxyl content or degree of esterification is an important factor for controlling the setting time of pectins, the sensitivity to polyvalent cations, and their usefulness in the preparation of low solid gels, fibres and film. It is determined by saponification of the pectin and titration of the liberated carboxyl groups.

Determination of MeO was done by using the Ranganna's method (1994). The neutral solution was collected from determination of equivalent weight, and 25mL of sodium hydroxide (0.25N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 minutes. After 30 minutes 25mL of 0.25N hydrochloric acid was added and titrated against 0.1N NaOH to the same end point as before like in equivalent weight titration.

Methoxyl content was calculated by using the following formula:

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

3.3.6 Total Anhydrouronic acid content (AUA)

Estimation of anhydrouronic acid content is essential to determine the purity and degree of esterification, and to evaluate the physical properties. Pectin, which is a partly esterified polygalacturonide, contains 10% or more of organic material composed of arabinose, galactose and perhaps sugars. Total AUA of pectin was obtained by the following formula (Ranganna's method, 1994).

anhydrouronic acid content was calculated according to the following foumula:

$$\% \text{ of AUA} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

Where, molecular unit of AUA (1 unit) = 176g
z = ml of NaOH from equivalent weight determination.
y = ml of NaOH from methoxyl content determination.
w = weight of sample

3.4 Microbiological analysis of jelly

3.4.1 Fecal coliform test procedure

Fecal coliform was performed as recommended by FDA, 2001 and test was inoculated in appropriate selective media for the quantitative determination of fecal coliform and *Escherichia coli* or *E. Coli* (Cody et al., 1999);

1. 0.2N NaOH (sodium hydroxide) solution was prepared by dissolving 8.0g of NaOH in deionized or distilled water and brought the volume up to 1L.
2. Rosolic acid solution was prepared by adding 0.1g of rosolic acid crystals to 10mL of 0.2N NaOH.
3. 5.210g of dehydrated mFC medium was taken in 100mL of deionized or distilled water into a 250mL flask.
4. Mixture was stirred well for several minutes to break up clumps and prevent medium from adhering to the flask.

5. Flask was placed in a heated water bath or on a hot plate and heated slowly to reach temperature at 90°C. Mixture was stirred constantly to prevent scorching. It was not autoclaved.
6. 1mL of rosolic acid solution was added per 100mL of medium with a clean pipette. Heating was continued until media was dissolved.
7. The medium was allowed to cool to a temperature of about 45-50°C. Then medium was poured 20 to 25mL in 90mm Petri dish bottoms.
8. When the medium had solidified (about 10 minutes), Petri dishes were closed by pressing firmly with the tops. Those plates were used after the medium had solidified.
9. Sample was spread on selective Petri dishes. Sterilized forceps were dipped in alcohol and was hold in Bunsen burner for speared out. Forceps was allowed to cool before use.
10. Petri dish was incubated for 24 hours at 44.5°C temperature.

3.4.2 Confirmation of *E. coli* using MacConkey Agar Media

1. 5.15g of dehydrated MacConkey Agar medium was taken in 100mL of deionized or distilled water into a 250mL flask.
2. Mixture was stirred well for several minutes to break up clumps and prevent medium from adhering to the flask.
3. Flask was placed in a heated water bath or on a hot plate and heated slowly to reach temperature at 90°C. Mixture was stirred constantly to prevent scorching. It was not autoclaved.
4. 1mL of rosolic acid solution was added per 100mL of medium with a clean pipette. Heating was continued until media was dissolved.
5. The medium was cooled to a temperature of about 45-50°C. Then medium was poured 20to 25mL in 90mm Petri dish bottoms.
6. When the medium had solidified (about 10 minutes), Petri dishes were closed by pressing firmly with the tops. These plates were suitable for using after the medium had solidified.
7. Then sample was spread on selective Petri dishes. Sterilized forceps was dipped in alcohol and was hold in Bunsen burner for speared out. Forceps was allowed to cool before using.

8. Petri dish was incubated for 24 hours at 37 ± 0.5°C.
9. Long-wave UV lamp was used for examine the colonies. Fluorescence indicates the presence of *E. coli*. Some UV lamps do not use the correct wattage and can give false results. UV lamps bulbs were checked in order to sure of specification.

3.4.3 Identification and Isolation of Salmonella spp.

Isolation of Salmonella was performed as recommended by FDA (Andrews et al., 1998).

Pre-enrichment:

1.0mL of the sample from the transport swab was inoculated in 9mL of buffered peptone water (Hi Media) and incubated at 37°C for 18h for pre-enrichment.

Selective Enrichment:

Further, for selective enrichment 0.1mL of the pre-enriched inoculum was transferred to 10mL of Rappaport-Vassiliadis broth (Hi Media) and incubated at 42°C for 24h.

Selective Plating:

After enrichment, a loopful (10µl) of inoculums was then streaked on xylose lysine desoxycholate (XLD) agar (Hi Media) and incubated at 37°C for 24h. The presumptive Salmonella colonies (4-5 colonies/plate) appearing slightly transparent red halo with a black center surrounded by a pink-red zone on XLD agar were screened further for its biochemical characterization.

Identification of Salmonella

The presumptive colonies of Salmonella were further subjected to biochemical tests viz., triple sugar iron (TSI), urease broth, indole, citrate test, lysine iron agar (LIA) test as per the standard test protocol described in Bacteriological Analytical Manual FDA.

3.5 Sensory evaluation

Nine points Hedonic rating test method as recommended by Joshi, (2006) was used for the purpose of sensory evaluation. This test measures the consumer's acceptability. The consumer acceptability of developed products was evaluated by a

testing panel. The panellists were untrained and selected from the students, teachers and employees of the Department of Food Processing and Engineering, Chattogram Veterinary and Animal Sciences University, Chattogram. The panellists (15) were asked to assign appropriate score to each product tested on a 1 to 9 point hedonic scale for characteristic colour, flavour, texture and overall acceptability of the samples of jelly.

The scale is arranged such that; 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, and 1 = Dislike extremely.

CHAPTER -4: RESULTS

4.1 Pectins yield in different extraction conditions

Extraction of pectins were carried out by water bath heating method, weighed malta peel powder (5g), distilled water (150mL) with addition of acids viz. Citric acid and Nitric acid. Different P^H was adjusted for maintaining 1.5, 2.0 and 2.5 P^H. The P^H of solution was adjusted in each extraction process according to different time, temperature and P^H treatment combination. From Malta peel powder extraction of pectin was done by using two different acids at different time (30 and 60m), temperatures (60, 70 and 80°C) and P^H(1.5, 2.0 and 2.5) total 36 times extraction i.e., 2(acids) * 18 (treatment combination) were performed.

Table 4.1: % yield of pectin obtained from malta peel with different extraction conditions.

Samp No.	**Type of acid**	**Time**	**Pectin % for P^H1.5**			**P^H2.0**			**P^H2.5**		
			60˚C	70 ˚C	80˚C	60 ˚C	70 ˚C	80 ˚C	60 ˚C	70 ˚C	80 ˚C
01	Nitric acid	30	18.7	20.9	22.1	23.2	26.4	28.9	15.4	16.3	17.8
02	Nitric acid	60	27.7	34.2	42.8	32.5	35.9	39.1	20.4	24.1	25.7
03	Citric acid	30	37.1 `	41.4	43.9	40.7	42.8	44.2	18.7	22.5	24.6
04	Citric acid	60	62.0	64.8	72.5	55.0	57.3	68.2	41.4	45.6	47.1

Extraction of pectin by citric acid and nitric acid was performed in Applied Chemistry and Chemical Engineering laboratory, CVASU. Table 4.1 shows the %yields of citric acid and nitric acid pectin samples. It can be seen from Table 4.1 that maximum

%yields of pectin were 72.5 and 42.4% for citric acid and nitric acid samples, respectively. On the other hand, minimum %yields of pectin were 18.7 and 15.4% for citric acid and nitric acid samples, respectively.

4.2 Effect of parameters on pectin yield

4.2.1 The effect of extraction time, temperatures and P^H of solution on pectin yield extracted from malta peel powder using nitric acid

The percentage yield of pectin extracted from malta peel powder using nitric acid at P^H1.5 for 30m at temperature 60, 70 and 80°C are 18.7, 20.9 and 22.1%, respectively. Whereas, at P^H1.5 for 60m at temperature 60, 70 and 80°C the percentage yield are 27.7, 34.2 and 42.8%, respectively (Figure 4.1).

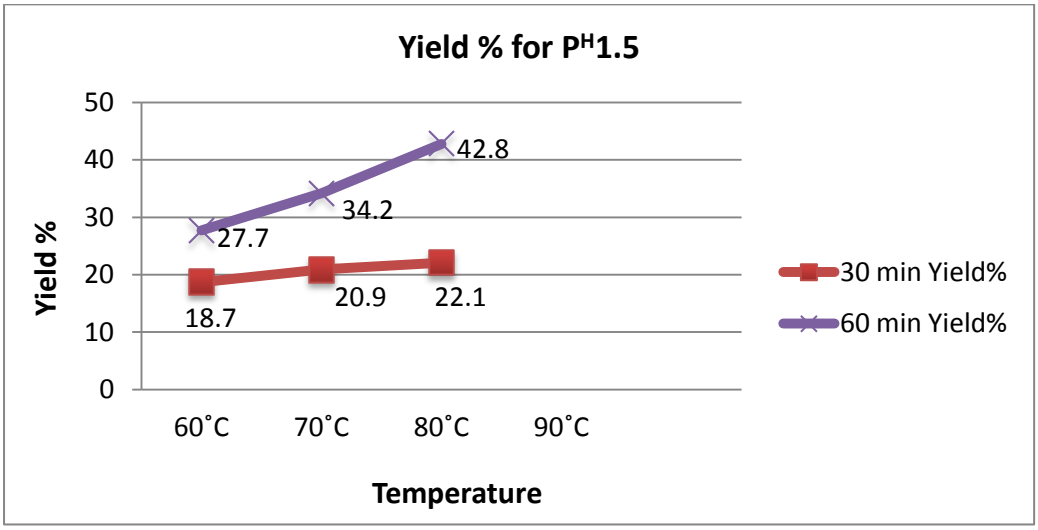

Figure 4.1: Effect of time and temperature on pectin yield at P^H1.5 using nitric acid.

At low P^H and higher extraction temperature there was sudden increased of percentage pectin yield. It was also found that the increased in the extraction time period also effect on the yield of pectin (Figure 4.1).

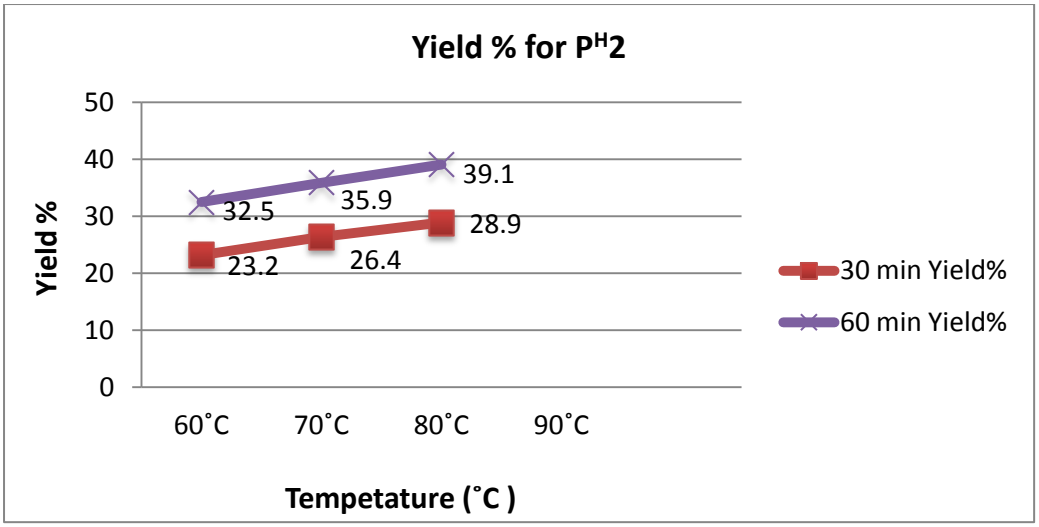

Figure 4.2: Effect of time and temperature on pectin yield at P^H2.0 using nitric acid.

The percentage yield of pectin extracted from malta peel powder using nitric acid at P^H2.0 for 30m at temperature 60, 70 and 80°C are 23.2, 26.4 and 28.9%, respectively. Whereas, at P^H2.0 for 60m at temperature 60, 70 and 80°C, the % yields are 32.5, 35.9 and 39.1%, respectively. At P^H2.0 the percent pectin yield was sudden increased from 30m to 60m at each temperature (Figure 4.2).

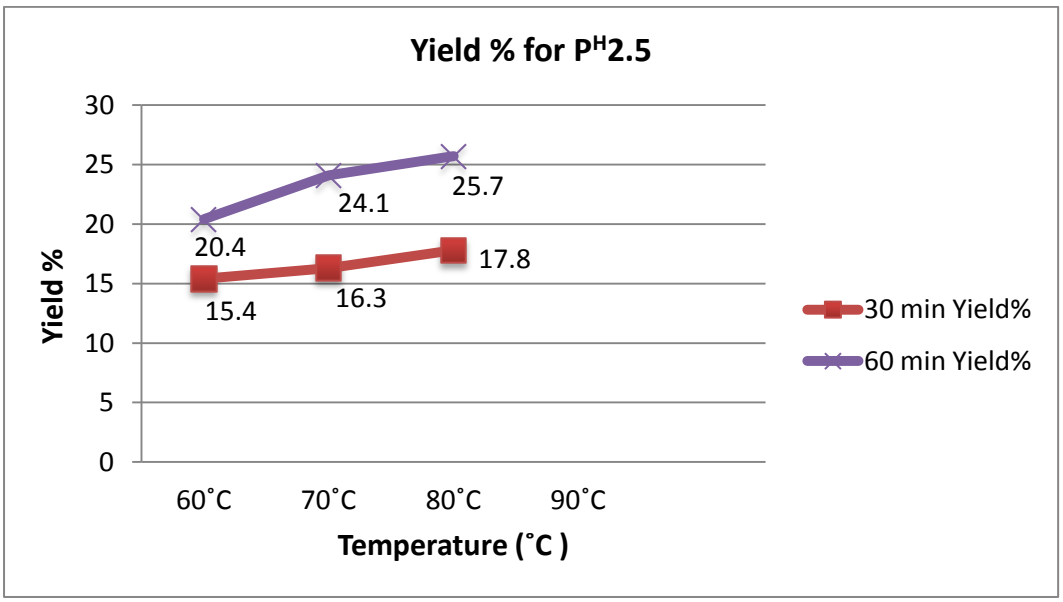

Figure 4.3: Effect of time and temperature on pectin yield at P^H2.5 using nitric acid.

The percentage yield of pectin at P^H2.5 extracted from malta peel powder using nitric acid for 30m at temperature 60, 70 and 80°C are 15.4, 16.3 and 17.8%, respectively. Whereas at P^H2.5 for 60m at the temperature 60, 70 and 80°C, the % yields are 20.4,

24.1 and 25.7%, respectively. At P^H2.5 there was increased on yield by increasing temperature and time of extraction (Figure 4.3).

4.2.2 The effect of extraction time, temperature and P^H of solution on pectin yield extracted from malta peel powder using citric acid

The percentage yield of pectin extracted from malta peel powder using citric acid at P^H1.5 for 30m at temperature 60, 70 and 80°C are 37.1, 41.4 and 43.9%, respectively. At P^H1.5 for 60m at temperature 60, 70 and 80°C, the % yields are 62.0, 64.8 and 72.5%, respectively. For 60m extraction, as the temperature increased, the yield was also increased and maximum yield was obtained at this particular treatment combination (Figure 4.4).

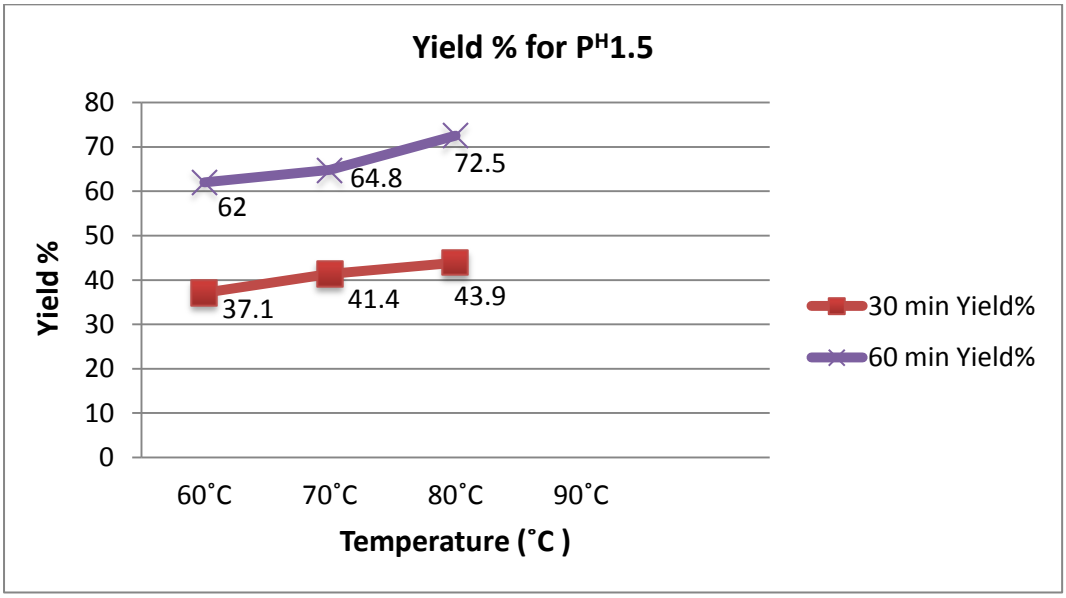

Figure 4.4: Effect of time and temperature on pectin yield at P^H1.5 using citric acid.

The percentage yield of pectin extracted from malta peel powder using citric acid at P^H2.0 for 30m at temperature 60, 70 and 80°C are 40.7, 42.8 and 44.2%, respectively. At P^H2.0 for 60m at temperature 60, 70 and 80°C, the % yields are 55.0, 57.3 and 68.2%, respectively. As the temperature raise due to increase the duration of time, resulted high pectin yield (Figure 4.5).

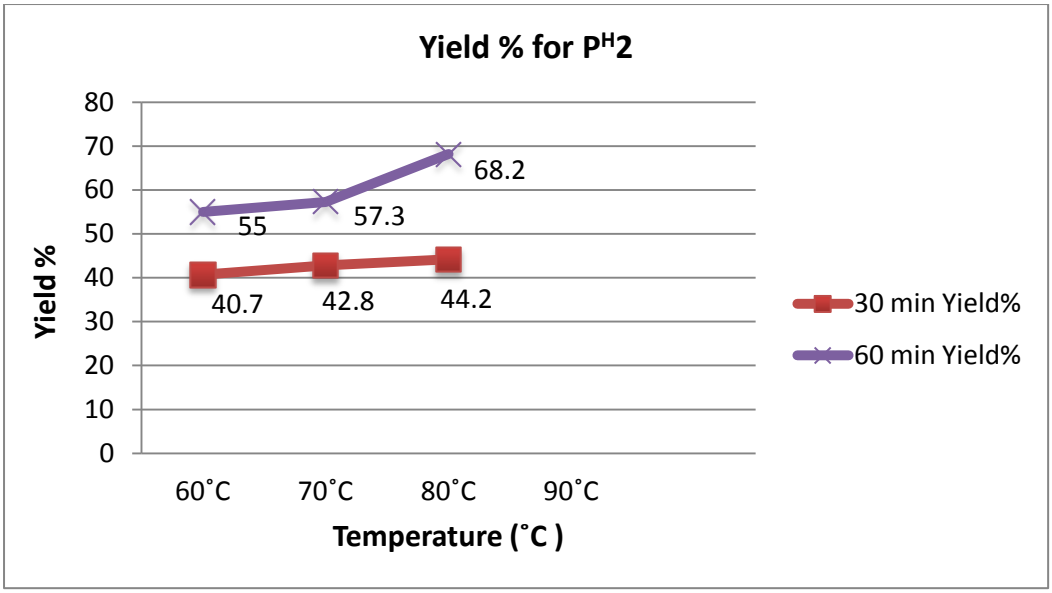

Figure 4.5: Effect of time and temperature on pectin yield at P^H2.0 using citric acid.

The percentage yield of pectin extracted at P^H2.5 from the malta peel powder by using citric acid for 30m at temperature 60, 70 and 80°C are 18.7, 22.5 and 24.6%. While at the same P^H for 60m the percent yields are 41.4, 45.6 and 47.1%, respectively (Figure 4.6).

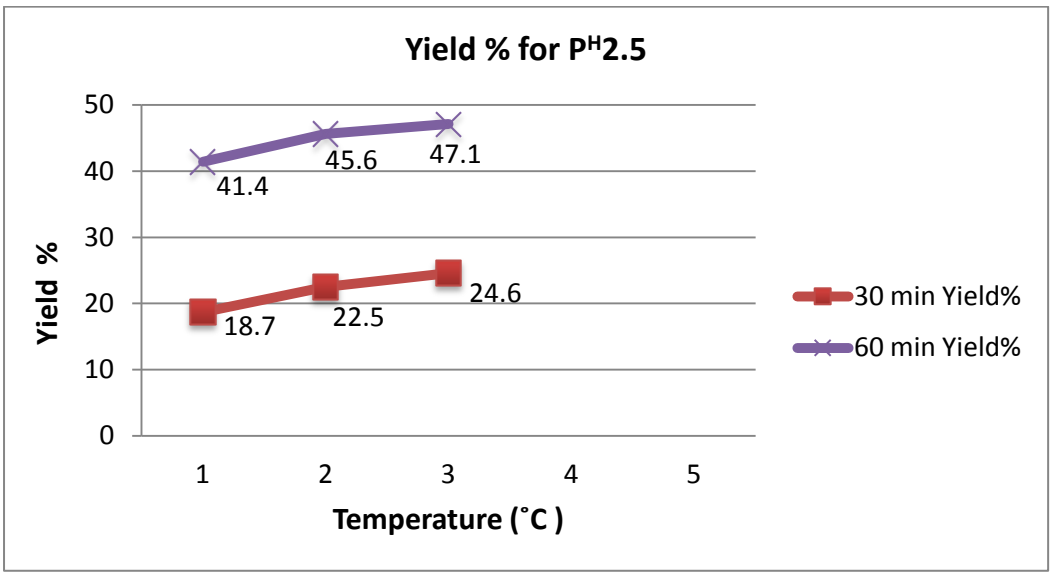

Figure 4.6: Effect of time and temperature on pectin yield at P^H2.5 using citric acid.

4.3: Physiochemical analysis of extracted pectin

Physiochemical analysis of pectin samples that are extracted by citric acid and nitric acid have been performed in Applied Chemistry and Chemical Engineering

laboratory, CVASU. Table 4.2 and table 4.3 show the laboratory test results of citric acid pectin samples and nitric acid pectin samples, respectively. These tables show the result of five parameters of physicochemical properties. It can be seen from Table 4.2 that maximum values of moisture content, ash content, equivalent weight, methoxyl content and anhydrouronic acid contents are 5.4%, 7.7%, 313.11, 6.4% and 93.07%, respectively, for pectins extracted by citric acid (Table 4.2). On the other hand, pectins extracted by using citric acid, the minimum values of moisture content, ash content, equivalent weight, methoxyl content and anhydrouronic acid contents are 5.0%, 7.1%, 312.02, 6.0% and 93.07%, respectively.

Table 4.2: Physiochemical test results for pectin (citric acid) samples

Sample	Moisture %	Ash %	Equivalent weight	Methoxyl content %	Anhydrouronic acid%
C1	5.3	7.1	312.62	6.0	92.57
C2	5.2	7.5	312.57	6.2	91.52
C3	5.2	7.3	312.51	6.2	90.89
C4	5.3	7.5	312.55	6.1	91.04
C5	5.4	7.2	312.02	6.3	91.52
C6	5.1	7.3	312.61	6.4	91.84
C7	5.2	7.5	312.57	6.2	91.52
C8	5.0	7.7	313.11	6.1	93.07

The results of physiochemical properties of pectin samples that are extracted from nitric acid are given in Table 4.3. The maximum values of moisture content, ash content, equivalent weight, methoxyl content and anhydrouronic acid contents are 7.8%, 3.9%, 832.82, 5.6% and 54.71%, respectively, for pectins extracted by nitric acid. On the other hand, minimum values of moisture content, ash content, equivalent

weight, methoxyl content and anhydrouronic acid contents are 7.2%, 3.2%, 832.31, 5.04% and 49.83%, respectively (Table 4.3).

Table 4.3: Physiochemical test results for pectin (nitric acid) samples

Sample	Moisture %	Ash %	Equivalent weight	Methoxyl content %	Anhydrouronic acid%
N1	7.2	3.3	832.31	5.32	51.34
N2	7.7	3.5	833.31	5.67	53.42
N3	7.6	3.5	833.33	5.27	51.04
N4	7.8	3.2	832.87	5.20	54.71
N5	7.5	3.7	833.52	5.12	50.33
N6	7.4	3.9	832.94	5.04	49.83
N7	7.6	3.5	833.33	5.27	51.27
N8	7.3	3.3	832.82	5.44	54.21

Pectin samples have been collected and tested in Applied Chemistry and Chemical Engineering laboratory, CVASU. The outputs of the laboratory test have been represented in the Figure 4.7 to Figure 4.11. Total 8 samples were collected and tested for two types of pectin samples. Sample number is assigned for each sample from C1 to C8. Figure 4.7 shows the moisture levels of ctric acid and nitric acid pectin samples, respectively. Detail results of each parameter are discussed in the next section.

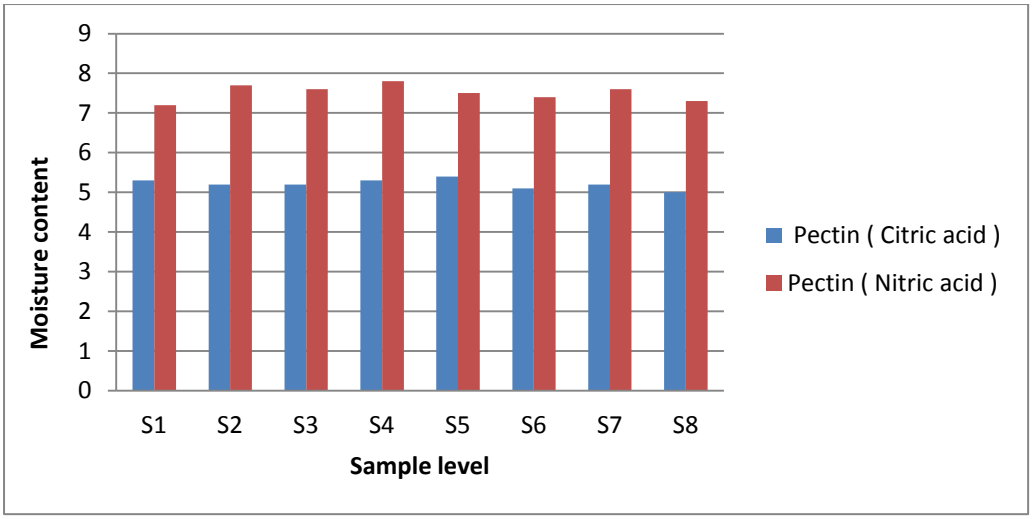

Figure 4.7: Moisture content (%) of citric acid and nitric acid pectin samples

Table 4.4: Descriptive statistics of Moisture content (%)

	Pectin (Citric acid)	Pectin (Nitric acid)
Mean	5.212	7.512
Standard Error	0.044	0.071
Median	5.2	7.55
Mode	5.2	7.6
Standard Deviation	0.124	0.203
Sample Variance	0.015	0.041
Kurtosis	0.146	-0.885
Skewness	-0.304	-0.223
Range	0.4	0.6
Minimum	5	7.2
Maximum	5.4	7.8
Sum	41.7	60.1
Count	8	8

Table 4.4 shows the descriptive statistics for citric acid and nitric acid pectin samples. The mean values for citric acid and nitric acid pectin samples are 5.212 and 7.512, respectively (Table 4.4). Standard deviation for citric acid and nitric acid pectins are 0.124 and 0.203, respectively. So, the moisture content dataset is more dispersive for the nitric acid pectin samples. Moisture value ranges from 5 to 5.4 for citric acid pectin samples. On the other hand, moisture value ranges from 7.2 to 7.8 for nitric acid pectin samples.

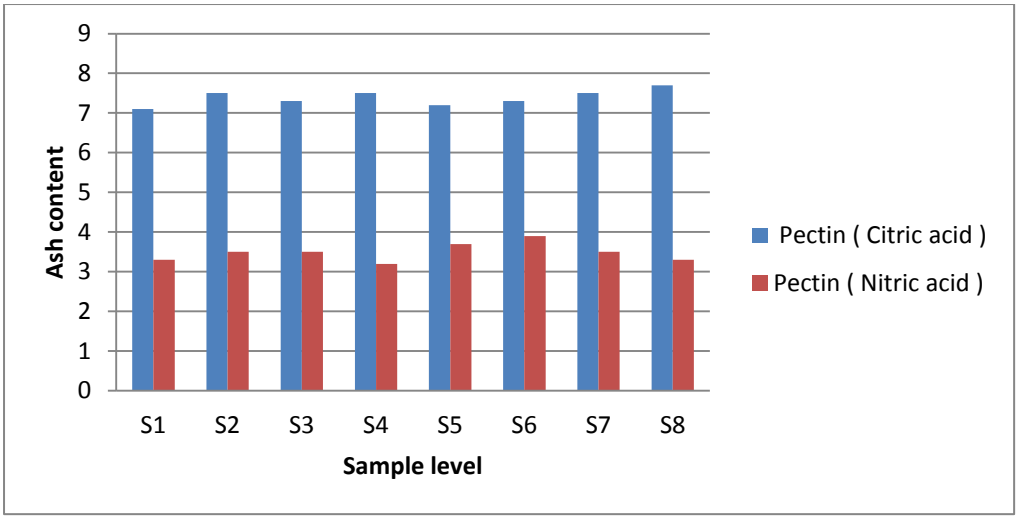

Figure 4.8: Ash content (%) of citric acid and nitric acid pectin samples

Table 4.5: Descriptive Statistics of Ash content (%)

	Pectin (Citric acid)	Pectin (Nitric acid)
Mean	7.387	3.487
Standard Error	0.069	0.081
Median	7.4	3.5
Mode	7.5	3.5
Standard Deviation	0.195	0.229
Sample Variance	0.038	0.052
Kurtosis	-0.666	0.078
Skewness	0.078	0.674
Range	0.6	0.7
Minimum	7.1	3.2
Maximum	7.7	3.9
Sum	59.1	27.9
Count	8	8

Table 4.5 shows the descriptive statistics for citric acid and nitric acid pectin samples. It can be observed from Table 4.5 mean values for citric acid and nitric acid pectin samples are 7.387 and 3.487, respectively. Standard deviation for citric acid and nitric acid pectins are 0.195 and 0.229, respectively. So, the ash content dataset is more dispersive for the nitric acid pectin samples. Ash value ranges from 7.1 to 7.7 for citric acid pectin samples. On the other hand, ash value ranges from 3.2 to 3.9 for nitric acid pectin samples.

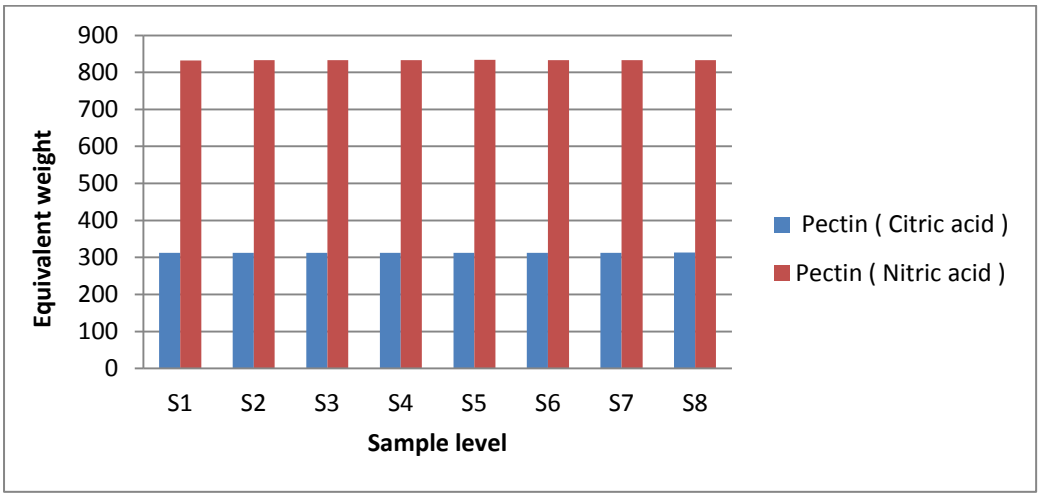

Figure 4.9: Equivalent weight of citric acid and nitric acid pectin samples

Table 4.6: Descriptive Statistics of Equivalent weight

	Pectin (Citric acid)	Pectin (Nitric acid)
Mean	312.57	833.053
Standard Error	0.103	0.139
Median	312.57	833.125
Mode	312.57	833.33
Standard Deviation	0.293	0.394
Sample Variance	0.086	0.155
Kurtosis	3.279	0.379
Skewness	-0.067	-0.841
Range	1.09	1.21
Minimum	312.02	832.31
Maximum	313.11	833.52
Sum	2500.56	6664.43
Count	8	8

Table 4.6 shows the descriptive statistics for citric acid and nitric acid pectin samples. It can be observed from Table 4.6 mean values for citric acid and nitric acid pectin samples are 312.57 and 833.053, respectively. Standard deviation for citric acid and nitric acid pectins are 0.293 and 0.394, respectively. So, the equivalent weight dataset is more dispersive for the nitric acid pectin samples. Equivalent weight value ranges from 312.02 to 31.11 for citric acid pectin samples. On the other hand, equivalent weight value ranges from 832.31 to 833.52 for nitric acid pectin samples.

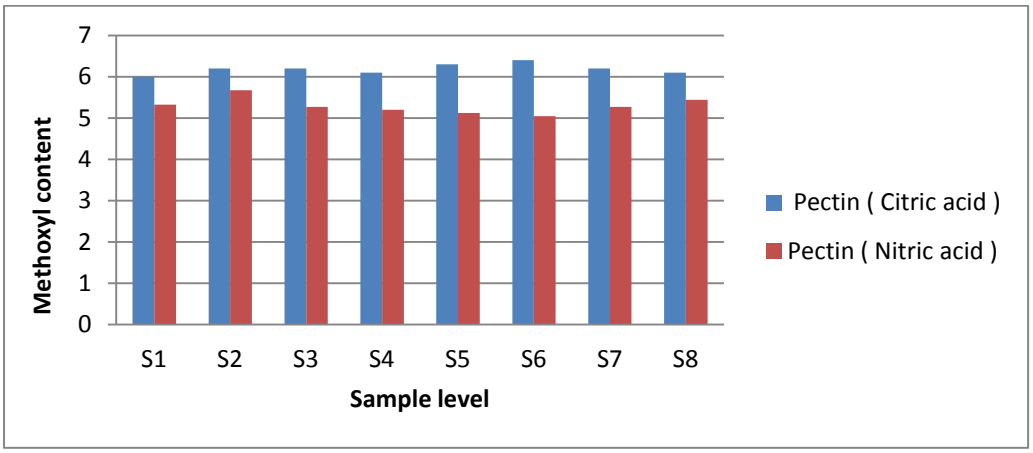

Figure 4.10: Methoxyl content (%) of citric acid and nitric acid pectin samples

Table 4.7: Descriptive Statistics of Methoxyl content (%)

	Pectin (Citric acid)	Pectin (Nitric acid)
Mean	6.187	5.291
Standard Error	0.044	0.069
Median	6.2	5.27
Mode	6.2	5.27
Standard Deviation	0.124	0.195
Sample Variance	0.015	0.038
Kurtosis	0.146	1.173
Skewness	0.304	0.914
Range	0.4	0.63

Minimum	6	5.04
Maximum	6.4	5.67
Sum	49.5	42.33
Count	8	8

Table 4.7 shows the descriptive statistics for citric acid and nitric acid pectin samples. The mean values for citric acid and nitric acid pectin samples are 6.187 and 5.291, respectively. Standard deviation for citric acid and nitric acid pectins are 0.124 and 0.195, respectively. So, the methoxyl content dataset is more dispersive for the nitric acid pectin samples. Methoxyl value ranges from 6 to 6.4 for citric acid pectin samples. On the other hand, methoxyl value ranges from 5.04 to 5.67 for nitric acid pectin samples.

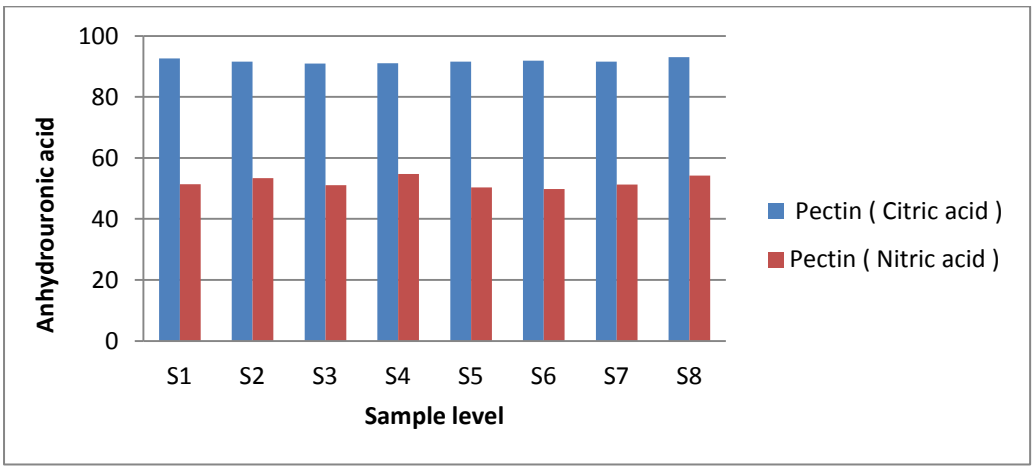

Figure 4.11: Anhydrouronic acid content (%) of citric acid and nitric acid pectin samples

Table 4.8: Descriptive Statistics of Anhydrouronic acid content (%)

	Pectin (Citric acid)	Pectin (Nitric acid)
Mean	91.746	52.018
Standard Error	0.261355925	0.649
Median	91.52	51.305
Mode	91.52	#N/A

Standard Deviation	0.739	1.837
Sample Variance	0.546	3.375
Kurtosis	0.066	-1.533
Skewness	0.879	0.485
Range	2.18	4.88
Minimum	90.89	49.83
Maximum	93.07	54.71
Sum	733.97	416.15
Count	8	8

Table 4.8 shows the descriptive statistics for citric acid and nitric acid pectin samples. The mean values for citric acid and nitric acid pectin samples are 91.746 and 52.018, respectively. Standard deviation for citric acid and nitric acid pectins are 0.739 and 1.837, respectively. So, the anhydrouronic acid content dataset is more dispersive for the nitric acid pectin samples. Anhydrouronic acid value ranges from 90.89 to 93.07 for citric acid pectin samples. On the other hand, anhydrouronic acid value ranges from 49.83 to 54.71 for nitric acid pectin samples.

4.4 Determination of microbial load

Contamination of fruit jelly by *Escherichia coli* (E. coli) and Salmonella is most common. Hence their presence in the final products is considered unfit for consumption. The microbiological analyzes are intended to ensure that the prepared jellies (Pineapple) has a higher hygienic and commercial quality. Microbiological analyses were performed for each of ten samples jellies. The result was found negative for all samples. The temperature and the cooking time can be at the origin of the hygienic quality of the product. The sugar content added in jellies prevented microbial spoilage.

4.5 Sensory evaluation of pineapple jelly

Prepared pineapple jellies are subjected to sensory evaluation test. The test has been performed by ten semi-trained panelists. The panelists comprised of female and male members who had previous experience on fruit jelly products evaluation. The evaluation of pipeline jellies was carried out on sensory attributes that include taste, flavors, mouthfeel, color, appearance and overall acceptability. This evaluation was performed at room temperature in the laboratory condition of department of applied chemistry and chemical engineering at CVASU. Each panelist scored samples independently and recorded the scores on the prescribed survey sheets provided. Panelists were served water and unsalted crackers to clean their mouths before tasting of each sample. The scale was arranged such that: Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slight = 4, Dislike moderately = 3, Dislike very much = 2, Dislike Extremely = 1.

This method does not, of course, reflect actual consumer perception, but it does strongly indicate attributes which a good quality product should possess (Sing et al., 2008).

Three jelly samples were developed from pineapple fruit, using two pectin extracted from malta peel using citric and nitric acid. Controlled jelly sample was developed without addition of pectin and compared the rest two samples with the controlled sample in terms of colour, taste, aroma, flavour, texture, appearance and overall acceptability. The jelly developed with addition of 0.5% pectin extracted from malta peel using nitric acid has scored 8.5 for taste, color, aroma and texture, 8.2 for flavor and appearance and 8.4 for overall acceptability. On the other hand, the jelly developed with addition of 0.5% pectin extracted from malta peel using citric acid has scored 8.5 for flavor and appearance, 8.2 for taste, texture, color and aroma, and 8.3 for overall acceptability.

CHAPTER -5: DISCUSSIONS

5.1 Effect of parameters on pectin yield

In the present study the highest yield (72.5%) was obtained from malta peel powder, extracted using citric acid, was the best for the extraction of pectin. This result was similar with the findings of Chang et al. (1994) who had compared the yields of pectin extracted from apple with different acids like hydrochloric acid, nitric acid and citric acid. Between the two strong acids, it was observed that there was a difference in pectin yield. Even though a low pH is necessary to improve the yield, the strong acid solution could lead to smaller pectin particles owing to partial hydrolysis. Consequently, pectin solubility would increase to the point that no precipitate was formed by the addition of alcohol. This could be the reason why the use of a stronger acid resulted in a lower pectin yield. Zhao (1995) reported that by using citric acid, nitric or sulphuric acid extractant, it has been shown that the type of acid strongly influences the macromolecular and gelling properties of isolated pectin, whereas, citric acid being the least pectin degrading (depolymerising and deesterifying) extracting agent. Therefore, it leads to pectin isolates with the best gelling properties.

On the other hand, extreme temperature and extraction time would lead to decomposition of pectin since pectin is composed of α-(1, 4) linked units of galacturonic acid or methyl ester. Turmucin et al. (1983) reported that the glycosidic bond is an ether bond that can go through hydrolysis reaction at the right conditions (80°C at P^H2 or at $P^H2.8$ for two hours). In this case, it is considered that by hydrolysis of high polymer of pectin molecules to low polymer leads to an increase of solubility in water, which makes it more difficult to separate pectin as a solid compound by the addition of ethanol. In the study carried out by Chang et al. (1994), the pectin yield increased initially but declined after 60 minutes of extraction. The decrease in pectin yield by the increase in extraction period may be due to the thermal degradation of the extracted pectin. The degradation is mainly caused by the depolymerisation mechanism of galacturonan chain of pectin, which is known as beta-elimination. Thus, the pectin cannot be recovered by precipitation with alcohol. The P^H during extraction was maintained at 1.5. They also reported that high concentration of hydrogen ions present in the solvent (at low P^H) stimulates the hydrolysis of protopectin. Protopectin is a compound formed by the combination of cellulose with

pectin molecules. During acid hydrolysis, the combination is split up to produce soluble pectin and cellulose by eliminating water molecules. As a result, protopectin becomes soluble pectin. The research of Joye D. D. et al. (2000) demonstrated that extraction under strong acidic conditions (below P^H2.0) was sufficient to extract the non-calcium sensitive pectin (NSCP) and the remaining pectin present in citrus peel, which is primarily calcium sensitive- pectin (CSP). Extraction under intermediate acidic conditions (approximately P^H3.0) was reported to extract only non-calcium-sensitive pectin. At lower P^H, the highly hydrated carboxylate groups are repressed in the larger hydrogen ion concentrations and therefore, converted into slightly hydrated carboxylic acid groups. The lost of charge is able to reduce the repulsion of the polysaccharide molecules which promote the gelation properties of pectin giving more precipitated pectin at lower P^H. Thus, the decreased in P^H is able to promote the liberation of pectin molecules from the peel during acid-washing stage because of the interaction of pectins to the hemicelluloses fractions are cleaved. Pectin yield is lesser in higher pH might be due to some pectin is still attached to the cell wall components, although pectin molecules can be partially solublised from plant tissues without degradation by weakly acidic aqueous solvents. The ethanol, as a surfactant solvent, significantly reduces the wetting angle of the plant tissues by modifying the drainage properties of the plant tissues. Consequently, the capillary pressure of the plant tissues is increased, and this condition causes an improvement in the penetration rate of the solvent.

5.2 Chemical composition of extracted pectin

5.2.1 Moisture content

The moisture content of pectin extracted from sweet lemon peel powder (SLPP) using citric and nitric acid was found to 5.21% and 7.51%, respectively. Similar results were found by Azad et al. (2014). The moisture absorbed by isolated pectin in this work was found to be in the range of 5.21 to 7.51%, which is slightly lower than that of 9.4-11.3% for commercial pectin, reported in the literature. The pectin is very hygroscopic, for this reason, it must be preserved in closed dry atmosphere. Literature data on the moisture content of pectin extracted from dragon fruit as well as different citrus peel like Kinnow, Musambi, Malta and Feutral lies in the range of 9.4- 11.3% (Turmucin et al., 1983).

5.2.2 Ash content

The ash content of pectin extracted from sweet lemon peel powder (SLPP) using citric and nitric acid was found to 7.38% and 3.48%, respectively, which is against 15.2% for commercial pectin. In literature, showed some parameter regarding different ash content of fruits as 6.9-11.6% for dragon fruit, on the other hand musambi, malta and feutral, orange peels contents 6.5- 8.9% ash. This parameter as reported in literature varies in a wide range depending on the method and the nature of the citrus fruits used for extraction. The upper limit of ash content for good-quality pectin is considered to be 10% from the view point gel-formation (Azad et al., 2014). Therefore, with respect to this parameter, the pectin isolated in this study may be considered to be of satisfactorily good quality.

5.2.3 Equivalent weight

Equivalent weight of pectin extracted from sweet lemon peel powder (SLPP) using citric and nitric acid was found to be 312.57 and 833.053, respectively. The present results has supported by Shaha et al. (2013). The equivalent weight of isolated pectin in his work was found to be 312.68 and 833.33 for citric acid and nitric acid, respectively. The over ripens lemon pomace extracted pectin showed lower equivalent weight (368.3) while the mature extracted sample showed the highest equivalent weight (1632.137) (Azad et al., 2014).This parameter as reported in literature varies in a wide range depending on the method and the nature of the citrus fruits used for extraction. High equivalent weight would have higher gelforming 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 (Rehman and Salaria, 2005).

5.2.4 Methoxyl content

The methoxyl content of pectin extracted from sweet lemon peel powder (SLPP) using citric and nitric acid was found to be 6.18% and 5.29%, respectively, this result was supported by Azad et al. (2014). The methoxyl content of isolated pectin in his work was found to be 6.32% and 5.49% for citric acid and nitric acid, respectively. The methoxyl content found for isolated pectin, which is against 3% for commercial pectin. Methoxyl content is an important factor in determining the gel formation

capacity. Methoxyl content is an important factor in controlling the setting time of pectins and the ability of the pectin to form gels. Spreading quality and sugar binding capacity of pectin are increased with increase methoxyl content (Azad et al., 2014). Based on methoxyl content value in this study indicates that sweet lemon peel pectin was categorized as high and low methoxyl pectin depends on reagent used.

5.2.5 Anhydrouronic acid (AUA) content

The AUA content of pectin extracted from sweet lemon peel powder (SLPP) using citric and nitric was found to be 91.74% and 52.01%, respectively, this results supported by Azad et al. (2014). The anhydrouronic acid content of isolated pectin in his work was found to be 91.52% and 51.42% for citric acid and nitric acid, respectively. The AUA indicates the purity of the extracted pectin and its value should not be less than < 65% (Food Chemical Codex, 1996). In this study the highest AUA content of pectin was found by using citric acid and the lowest using nitric acid (52.01%). Low value of AUA means that the extracted pectin might have a high amount of protein, starch and sugars in the precipitated pectins.

5.3 Sensory analysis of jelly developed by using extracted pectin

The jelly was developed from 170mL fruit extract for each sample, added 0.52g of citric acid for each sample and from this amount of acid, little amount was added during fruit extract and the remaining citric acid was added after addition of 127.5g of sugar for each sample and alcohol test was done to check the pectin content in fruit extract, it was found in the ranged of moderate pectin content as the clot showed less firm and fragmented. The amount of pectin was fixed to 0.5% for each sample, i.e 0.42g. After boiling testing was done by measuring temperature up to 105°C and checked °brix using refractometer till the TSS attend 65°brix. Three jelly samples were developed from pineapple fruit, using two pectin extracted from malta peel using citric and nitric acid. Controlled jelly sample was developed without addition of pectin and compared the rest two samples with the controlled sample in terms of colour, taste, aroma, flavour, texture, appearance and overall acceptability. Organoleptic score was taken from the 9 panelist using 9-Point Hedonic Scale sensory evaluation card.

The jelly developed with addition of 0.5% pectin extracted from malta peel using nitric acid has scored 8.5 for taste, color, aroma and and texture, 8.2 for flavor and appearance and 8.4 for overall acceptability. Appearance was not good as compared to control sample, it was found to be little foam suspended. Jelly gives smooth texture. The taste of jelly was good but it gave a little strong flavor, which was found to be not much desirable. The jelly developed with addition of 0.5% pectin extracted from malta peel using citric acid has scored 8.5 for flavor and appearance, 8.2 for taste, texture, color and aroma, and 8.3 for overall acceptability. This jelly developed from pineapple fruit extract with addition of 0.5% pectin extracted by using citric acid was found to be the less in score. Here for this particular sample the color, taste, aroma and texture is less as compared to the control sample. This jelly gave a little strong aroma somewhat citric acid flavor, this may be due to addition of pectin extracted from sweet lemon using citric acid, so the aroma of citric acid from pectin might be influenced on the jelly. The texture of this jelly was good in terms of spreadability.

CHAPTER -6: CONCLUSIONS

This research emphasized on pectin extraction and characterizations from Sweet lemon (malta) peel. In general, the research had been divided into four parts namely effect of reagents on pectin yield, effect on pectin yield by different parameters, characterization of pectin and preparation of jelly using extracted pectin, microbial and sensory evaluation of jelly. The results indicated that different extractants, P^H, extracting temperature and time effect on the extraction yield. The best condition were, extracting temperature at 80°C at P^H1.5 for 60m and using citric acid as the extracting solvent. This gave a yield of 72.5%. From the results obtained, sweet lemon peel gives a significant amount of pectin whereby it can be considered in commercial production of pectin alongside with other citrus sources.

CHAPTER -7: RECOMMENDATIONS AND FUTURE PERSPECTIVES

These studies have been concluded with good findings in the new area of developing low cost technologies for extraction of pectin. It is also resulted with its commercial value and better marketability. Modern food industries can adopt the procedure for medium and large scale pectin production. On the basis of present investigation, the following suggestions and prospects are made for the further research work.

- The present studies may be repeated for confirmation of the experimental findings.
- The composition may be modified further and may try for extracting pectin with various procedures with different extraction conditions.
- This study can also be repeated with addition of other acidic condition.
- The findings will be helpful from therapeutic point of view for people suffering from diabetics because pectin is a digestive aid and reduce the absorption of glucose in the serum of diabetics.
- On the other hand, it will be helpful from economic point of view. If we can extract pectin from fruit by products it will be lower cost of pectin and ultimately it will be cost effective for production of jelly.
- Such types of research should be done for other fruits like lemon, pumpkin etc.
- Although the sample size was sufficient to perform statistical comparisons between analytical data. Our conclusion should be considered with caution because of the small number of analyzed samples and results would need to be confirmed with another larger study.
- Sufficient steps should be taken to enrich commercially available jellies with more nutritional value.
- Necessary steps should be taken to control quality and value of commercial jellies.

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Appendix

Appendix A: Photo gallery

Fig A1: Drying of malta peel

Fig A2: Grinding of dried malta peel

Fig A3: Extraction of pectin from malta peel using citric acid and nitric acid

Fig A4: Extracted pectin from citric acid

Fig A5: Extracted pectin from nitric acid

Fig A6: Drying of extracted pectin

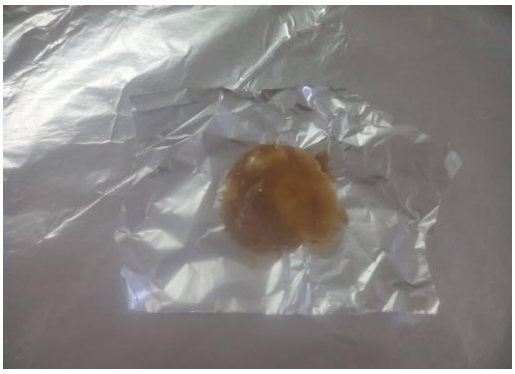

Fig A7: Dried pectin

Fig A8: Pineapple sample

Fig A9: Preparation of pineapple jelly

Fig A10: Developed pineapple jelly by extracted pectin

Appendix B: Tasting of Pineapple jelly (Hedonic Rating Test)

Name of Taster..... Date: .....

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as colour, flavour, texture and overall acceptability. Use the appropriate scale to show your attitude by checking at the point that best describe your sense and feeling about the sample please give a reason for this attribute. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us.

For Colour/Flavour/Taste/Overall Acceptability

Hedonic	Colour			Flavour			Texture			Acceptability		
	A	B	C	A	B	C	A	B	C	A	B	C
Like extremely												
Like very much												
Like moderately												
Like slightly												
Neither like nor dislike												
Dislike slightly												
Dislike moderately												
Dislike very much												
Dislike extremely												

The scale is arranged such that; Like extremely =9, Like very much =8, Like moderately =7, Like highly =6, Neither like nor dislike =5, Dislike slightly =4, Dislike moderately =3, Dislike very much =2, and Dislike extremely =1.

Appendix C: Rating Score

Brief bio-data of the student:

Afruz Jannat Laboni passed the Secondary School Certificate Examination in 2009 and Higher Secondary Certificate Examination in 2011. She also received Bachelor of Science (Hons.) in Food Science and Technology degree from Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Khulshi-4225, Chattogram. Now, she is a candidate for the MS degree in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Engineering of same faculty. Her research interests are in the areas of development of chemical ingredient in processing and preservation of food. She is also interested in studying the health implications of using additives and preservatives during food processing