ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIOENGINEERING

PECTIN DEVELOPMENT FROM CITRUS PEELS AND THE EFFECT OF PROCESSING CONDITIONS ON THE EXTERACTION

By:

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A Thesis Submitted to Addis Ababa Institute of Technology, School of Chemical and Bio Engineering in Partial Fulfillment of the requirement for Degree of Masters of Science in Chemical Engineering (Process Engineering).

Advisor: ADAMU ZEGEYE (Associate Prof.)

JULY 2016

ADDIS ABABA, ETHIOPIA

ADDIS ABABA UNIVERSITY

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I hereby declare that the thesis is based on my original work except for flotation's and citations which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other schools at Addis Ababa University or other institutes.

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Acknowledgements

First of all, I would like to thank God for giving me health, strength, and patience to accomplish this thesis work successfully. I wish to express my genuine gratefulness to my advisor, Associate professor Adamu Zegeye, whose encouragement, guidance, motivation, intelligence, and expectations were crucial to my achievement.

My appreciation goes to Chemical Engineering Laboratory supervisors in Addis Ababa University Mr. Hinsa, Samson, Akililu, and Azeb are to mention few. Without them the work could have not been performed within the given time.

I would also like to acknowledge the Upper Awash Agro Industry Enterprise workers for their invaluable support and provision of lemon varieties for this study that making my laboratory work easier. My special appreciation goes to Mr. Jela, Mr. Habtamu and drivers who sacrificed their time and energy for me to get the research varieties.

It is my pleasure to express my sincere appreciation to my family. Their encouragement in my small achievements has always been a driving force for me. They were with me when I am in need of their support. I am sure that I would not have been able to achieve this without their support, and prayer.

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ACRONYMS

ANOVA;	Analysis of Variance
AOAC;	Association of Official Analytical Chemists
AUA;	Anhydrouronic acid
CPW;	Citrus Processing Wastes
CRD;	Completely randomized design
DC;	Direct Cost
DE;	Degree of Esterification
FAO;	Food and Agricultural organization
FCI;	Fixed Capital Investment
HG;	Homogalacturonan
HM;	High Methoxyl
IC;	Indirect Cost
ITC;	International Trade Commission
LM;	Low Methoxyl
MC;	Manufacturing Cost
NPV;	Net percent Value
R^2;	Coefficient of determination
RoR;	Rate of Return
TCI;	Total Capital Investment
TMC;	Total Manufacturing Cost
UAAIE;	Upper Awash Agro Industry Enterprise
UN;	United Nation
US;	United States

ABSTRACT

The aim of this study was to extract pectin from two different varieties of citrus fruit namely Mexican lime (Citrus aurantifolia Swingle) and Lisbon lemon (Citrus limon "Lisbon") and to investigate the effect of processing conditions on the process of extraction. Research samples were collected from Upper Awash Agro-Industry Enterprise. The raw material was prepared and pectin was extracted using nitric acid at different pH and temperature. Pectin was extracted from two varieties, at three different temperatures (40 °C, 60 °C, & 80 °C), and pH (1.0, 2.5, & 4.0). The experiments were carried out in water bath for two hours. Design-Expert 7.0.0 at three-level two-factor general factorial design was applied. A total of 27 experiments were conducted for each variety at various extraction conditions. From the analysis of experimental data the interaction effects were studied and the optimal process conditions, maximizing the percentage yield were found. Using nitric acid, the yield of pectin for Mexican variety varies from 4.6% -19.6% and from 5.4%-21% for Lisbon variety. It was also observed that with a decrease in pH the pectin yield increased. Similarly with an increase in extraction temperature, the pectin yield also increased. Maximum yield of 19.6% was obtained at pH 1.0 and temperature of 60 °C for Mexican variety whereas maximum yield of 21% was obtained at pH 1.0 and temperature of 60 °C for Lisbon variety. The best condition for extraction using nitric acid was at 60 °C for 2 h at pH 1.0. The pectin obtained from these methods was compared in terms of yield, physicochemical properties and chemical structure. Functional groups present in the pectin were investigated using Fourier Transform Infrared Spectroscopy (FTIR). The characterization of the extracted pectin was done by calculating the ash content, moisture content, equivalent weight, methoxyl content, anhydrouronic acid content and degree of esterification. Methoxyl content, anhydrouronic acid, and degree of esterification of extracted pectin varied from 3.41-4.15%, 63.71-74.27%, 27.62-30.45%, and 6.14-6.94%, 51.04-67.89%, 51.34-77.24% for Mexican and Lisbon varieties respectively. Preliminary feasibility study indicated that production of pectin from lemon peel is feasible with Birr 3.52 million TCI, with 17.75% ROI overall after tax, Birr 624,637 Npav, 2 year and 6 months payback period. Therefore, the above figures show industrial production of pectin from lemon peel is feasible and it would save valuable foreign exchange by reducing pectin imports.

Key words: Pectin; Citrus peel; Processing conditions: Extraction; Yield

1. INTRODUCTION

Worldwide, annual production of all types of citrus fruit now stands at over 110 million tons, covering an area of nearly 18.7 million acres. Around 60% of all citrus production is oranges and 23% tangerines, mandarins, clementines and satsumas. Around 13.7 million tons of lemon and limes and 4.4 million tons of grapefruit and pomelos are also produced (Yara, 2016).

The production of citrus juice generates a large quantity of citrus processing waste (CPW), with around 44–60% of the mass of processed fruit ending up as CPW (Widmer *et al.*, 2010). Thus, approximately 5.4 million tonnes of wet CPW is generated annually in the world by the lemon/lime processing industry alone.

Citrus waste contains 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 (Abdel *et al*., 2013).

Pectin is complex mixture of polysaccharides and although pectin occurring in the primary cell walls of terrestrial plants, a high value functional food ingredient. It consists of a linear backbone of -(1-4)-D-galacturonic acid residues partially esterified with methanol, with periodic interruptions to L-rhamnose residues that make backbone irregular and with some other neutral sugars present as side chains. The general makeup of the pectin content varies with the ripening of the fruit.

Pectin is produced commercially in the form of white to light brown powder, mainly extracted from citrus fruits and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber. Several studies have reported novel pectin usages, like biodegradable water-soluble films, bulking agents, coating agents, chelators, emulsifiers and duration and viscosity modifiers the amount, structure and chemical composition of the pectin differs between plants, within a plant over time and in different parts of a single plant.

Although pectin is occurs commonly in most of the plant tissues, the number of sources that may be used for commercial manufacture of pectin is limited. This is because the ability of pectin to form a gel depends on molecular size and the degree of the esterification (DE).

At present, commercial pectins are almost exclusively pectin derived from citrus peel or apple pomace, both of which are by-products of juice manufacturing units. Apple pomace contains 10-15% of pectin on a dry matter basis. Citrus peel contains relatively higher, i.e. 20-30% of pectin as compared to that of apples (Kanmani *et al*., 2014).

The aim of this paper is to investigate extraction of pectin from peels of two different varieties of citrus fruit and to obtain optimum conditions for maximum extraction of pectin using design expert software general factorial method, and to study the physicochemical properties of the pectin extracted from the two fruit peels.

1.2 Statement of the problem

The world population increment has caused a growing concern about the environment, especially in industrial and agricultural activities, with several studies and investments in clean technologies, resource efficiency, recycling and waste reuse.

One of the major problems challenging the food industry throughout the world is how to make full utilization of the waste material. These waste materials, such as citrus peels and other residues from 60% to 65% of bulk citrus fruit after processing such residues are normally thrown as waste during processing (Kanmani *et al*., 2014).

Citrus waste contains 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 (Kanmani *et al*., 2014).

When lemon/lime juice producer is done squishing his lemons, a lot of lemon/lime peel is left unused. This peel is considered by some farmers to be waste and is used to feed the cattle or perhaps as fertilizer. This peel is the raw material for extraction of essential oil as well as pectin. By using a range of devices, pectin can be extracted from the peel, which can then be sold. This way the lemon peel is used for a better purpose.

Currently, the markets for pectin consume only a relatively small fraction of the CPW. For example, the world market for pectin, which is used as a gelling agent in the food industry, was estimated as 80,000 tonnes per year (Summer, 2016). Whereas the CPW generated annually by the lemon/lime processing industry could produce approximately 219,200 tonnes of pectin. In other words, over 90% of the CPW produced by the lemon/lime processing industry is in excess of the world pectin market. This excess CPW could be used for animal feed. However, this is seldom economic, due to the high energy requirements for the processes of drying and milling that are necessary to transform the CPW into a meal that is appropriate for adding to animal feed (Grohman *et al*., 2013). As a result, the CPW is sometimes simply discarded. It is therefore highly desirable to find new ways of converting CPW into useful products.

In Ethiopia, there are several farms which produce lemon/lime at large scale the total amount of fruits being produced from this farms is around 77,087 tons (CSA, 2013) without including small farms and household farms. The peel of all the lemon/lime is either damped to the environment or given to animals. Therefore this research focuses on CPW's as a potential raw material for production of pectin.

1.3 Objectives of the study

1.3.1 General objective

The main objective of this research is to extract pectin from lemon peel and to study the effect of pH, and extraction temperature on the yield of pectin.

1.3.2 Specific objectives

- To investigate the effect and interaction of temperature, and pH on the extraction of pectin.
- To generate the model equation for independent variables that maximizes the degree of extraction of pectin.
- Characterization of the physicochemical properties (Moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid, and degree of esterification) of pectin extracted.
- To compare the characteristics of two different pectins and see which one is more suitable for commercial production?
- Conducting preliminary feasibility study for extraction of pectin.

2. LITERATURE REVIEW

2.1 Pectin

The term pectin was first described and isolated by Henry Braconnot in 1825. Pectin is a polysaccharide, naturally occurring substance present in all plant tissue. Pectin exists in varying amounts in fruit cell walls and has important nutritional and technological properties. In the cell walls they serve as one of the main agents cementing the cellulose fibrils and may be linked covalently to other polymers. Intracellular pectin's provide the channels for passage of nutrients and water (Srivastava and Malviya, 2011).

Pectin is the methylated ester of polygalacturonic acid, which consists of chains of 300 to 1000 galacturonic acid units. It is located in the cell walls and the middle lamellae of plants (Kalapathy and Proctor, 2001). In oranges, for example, the pectin is found in the white spongy albedo of orange peels. Pectin is a natural food additive used extensively in the food industry. The world market demand for pectin is in excess of 30,000 tons annually and is growing by about 4% to 5% per annum. The peel, after pectin extraction, could still be used as a high-protein stock feed in dry form, increasing the potential return for the orange juice industry and reducing the pollution load on the environment (Yeoh *et al*., 2008).

2.2 Sources of pectin

Pectin is a complex mixture of polysacharides that makes up about one third of the cell wall of dry substance of higher plants. Much smaller proportions of these substances are also found in the cell walls of grasses. The highhest concentration of pectin are found in the middle lamella of cell wall, with a gradual decrease as moving through the primary wall gradual decrease as moving through the primary wall toward the plasma membrane. Although pectin occurs commonly in most of the plant tissues, the number of sources that may be used for the commercial manufacture of pectin is limited. This is because; the ability of pectin to form gel depends on the molecular size and degree of estrification (DE). The pectin from different sources does not have the same gelling ability due to variations in numerous numbers of parameters. Therefore, detection of a larege quality of pectin in a fruit alone is not in itself enough to qualify that fruit as a source of commercial pectin. At present, commercial pectins are almost exculisively derived from citrus peel or apple pomace, both of which are by-products from juice

manufacturing units. Apple pomace contains 10-15% of pectin on a dry matter basis. Citrus peel contains relatively higher i.e. 20-30% of pectin as compared to that of apple (Srivistava and Malviya, 2015).

High methoxyl pectins are mainly used as gelling agents in fruit-based products, especially in the manufacture of jams and fruit preservatives. Other food applications include milk desserts, fruit preparations for yoghurt and tart glazings, confectionery jellies, heat-resistant bakery jams, stabilization of acidified milk products, fruit juices, or soft drinks. Low methoxyl pectins are used to prepare gels with a reduced level of dissolved solids, and are of great interest because of their reduced calorific value. Applications in food industry are diversified, including jams and jellies of low-sugar content, dairy desserts, where usually addition of calcium salt is not needed, fruit gels for use in ice cream, as food coatings, and as thickening agents of syrups for fruit and vegetable canning, among many others (Stephen *et al*., 2006).

De-esterification at the industrial level is generally carried out on the precipitated HM pectin in alcohol dispersion. The LM pectins are generally obtained by controlled acid de-esterification, or by alkali de-esterification. The use of enzymes to extract pectins from vegetable raw materials and also to promote de-esterification reaction has been suggested, but it is still rarely used at the industrial level because of economic constraints. If the base used is ammonia, a different type of LM pectin is obtained in which some carboxylic groups are amidated. The presence of some carboxyl groups in the amide form (typically around 15–18%) allows this type of pectin to tolerate more variation in calcium content and to originate gels with a higher degree of thermo reversibility (Stephen *et al*., 2006).

2.3 Structure

Pectin is a polysaccharide, composed of different sub-structural entities that vary with the extraction methodology, raw material, location, and other environmental factors. The linear backbone of the pectin polymer is called homogalacturonan, and is built by sequences of α (1→ 4) linked D-galacturonic acid residues. These building blocks of polygalacturonic acid can be esterified and present as the methylesters and the free acid groups. They may be partly or fully neutralized with cations such as sodium, potassium, calcium, or ammonium. The ratio of esterified galacturonic acid groups to total galacturonic acid groups is termed the degree of

esterification (DE). It is possible that the homogalacturonan is fully methyl esterified when synthesized in the plant, but the highest DE that can be achieved by extraction of natural raw material is approximately 80%. Pectin with DE from 5%-75% is produced by controlled de-esterification in the manufacturing process. A DE of 50% conventionally divides commercial pectin products into HM and LM pectin. The DE has a vital influence on the properties of pectin HM pectin requires a minimum amount of soluble solids (SS) and a pH around 3.0 or lowers in order to form gels. LM pectin requires the presence of a controlled amount of calcium, or other divalent cations, to form a gel by "bridging" between adjacent chains and therefore is more flexible with respect to sugar and/or acid (Miceli-Garcia, 2014). Pectins are complex polysaccharides mainly composed of 1-4 linked α-D-galacturonic acid (Fig. 2-1) and found in middle lamella of plant cell membrane (Garna *et al*., 2004).

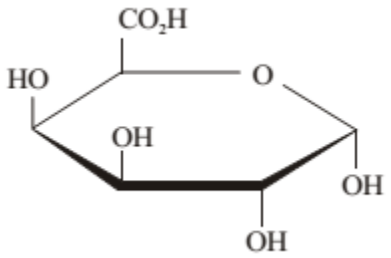

Figure 2-1 Structure of pectin

D-Galacturonic acid is a sugar acid, an oxidized form of D-galactose and is present as polygalacturonic acid in pectin as shown in (Fig. 2-2). It has an aldehyde group at C1 and a carboxylic acid group at C6. Other oxidized forms of D-galactose are D-galactonic acid (carboxylic group at C1) and meso-galactaric acid (mucic acid) (carboxylic groups at C1 and C6). It is also a uronic acid or hexuronic acid (khan *et al*., 2015).

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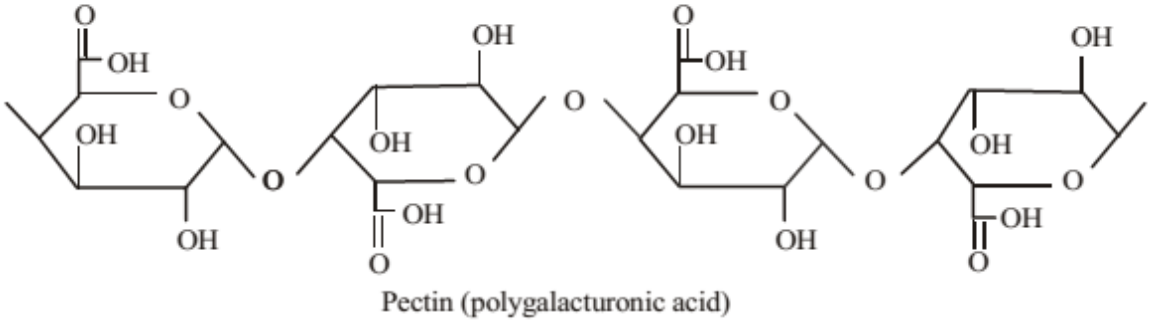


Figure 2-2 Structure of polygalactronic acid

The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts with sodium, potassium or calcium. The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5% the salts are called pectates, the insoluble acid form, pectic acid. Some of its galacturonide units are esterified as methyl galacturonate. One of the most important properties of pectin is gelling. The Degree of Esterification (DE) affects the gelling properties of pectin. The structure shown in Fig. 2-3 has three methyl ester forms (-COOCH3) for every two carboxyl groups (-COOH), hence it is has a 60% degree of esterification, normally called a DE-60 pectin. High Methoxy (HM) pectins have the ability to form gels with sugar and acid, so-called low water activity gels or sugar-acid- pectin gels (khan *et al*., 2015).

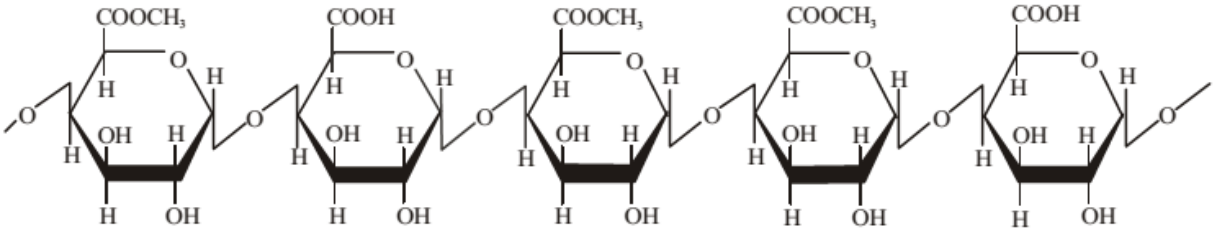


Figure 2-3 Pectin a polymer of galactronic acid

Such a gel is considered as 2-dimensional network of pectin molecules in which the solvent (water) with the co-solutes sugar and acid are immobilized. This results in a system resisting deformation and showing a stress-strain relationship for small deformation. The buildup of the 3-dimensional network is based on the formation of junction zones in which there are chain associations stabilized by hydrogen bonding between undissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters. The gelation mechanism

of pectin is mainly governed by their Degree of Esterification (DE). For the low methoxyl pectins, denoted LMP (DE<50%), gelation results from specific non-covalent ionic interactions between blocks of galacturonic acid residues of the pectin backbone and with divalent ions such as calcium. The affinity of pectin chains towards calcium is known to increase with decreasing degree of esterification or ionic strength and with increasing polymer concentration. Besides the influence of the charge density of the polygalacturonate chain, the distribution pattern of free and esterified carboxyl groups has an important effect on the strength of calcium binding. Due to this property, pectin has wide applications in a variety of food formulations as jellying and thickening agent. Pectin is a natural food additive used extensively in the food industry as a gelation agent. It is also being recommended for use as fat replacer. The world market demand for pectin is increasing faster and growing by about 4-5% per annum (Garna *et al*., 2006). On account of its ever-increasing use and demand pectin has become an indispensable ingredient in food industry. Major sources of pectin are apple pomace and citrus peel. Dried citrus peel has about 30% pectin (khan *et al*., 2015).

2.4 Gelling properties and applications

2.4.1 Low methoxy pectin gels

Calcium induced gelation is predominant in low methoxy pectin gels. Gelation is due to the formation of intermolecular junction zones between the 'smooth' HG regions of separate polymers. The nature of the interaction, although known to be electrostatic to some extent, is still debated. Gel forming ability decreases with degree of methoxylation and some blockwise distributions of carboxyl groups are very sensitive to calcium presence. The effect of calcium is decreased by the acetylation of the pectin. Amidation, conversely, improves the gelling ability of LM pectins and are less prone to precipitation by high calcium levels. The modification of the hydrogen bonding nature of the polymer by the addition of amide or acetyl moieties indicates that the 'egg-box' mechanism of gelation may not apply for all situations of calcium induced low methoxy pectin gelation.

2.4.2 High methoxy pectin gels

Jams and preserves are of course the main use of industrially extracted pectins. High dissolved sugar and acid conditions ensure that chain-chain interactions dominate over chain-solvent

interactions. Most chain-chain interactions in these systems are not based on electrostatic interactions and so the other hydrophobic and hydrogen bonding effects exert most influence. High sugar conditions create low water activities which can be mimicked by other solutes with the same resulting gels. This is reflected in the subdivision of HM pectins into rapid-set pectins of DM~77 to slow-set with DM~60. The larger hydrophobic element in HM pectins allows for rapid arrest of the systems (Sharma *et al*., 2006).

Many factors influence the conditions of gel formation and the gel strength achieved. The major role is played by the pectin molecules, their chain length, and the chemical nature of the junction zones. Under equal conditions gel strengths increase with the molecular weight of the pectin used, and any treatment depolymerising the pectin chains is reflected in weaker gels. Rupture strength depends on the number of junction zones per single long chain molecule whereas for rigidity the number of junction zones per unit gel volume plays a greater role. Acetyl groups prevent gelation and the DM within the group of high methoxyl pectins determines the setting temperature of a gel (Sharma *et al*., 2006).

Powdered HM-pectins slowly lose their ability to from gels if stored under humid or warm conditions while LM-pectins are more stable and loss should not be siginificant after one year storage at room temperature (Srivistava and Malviya, 2015).

The manufacture of pectin typically comprises extraction, purification, concentration and drying. The use of a suitable method for pectin extraction is significant in order to maximize its yield and quality. The literature concerning most commonly used methods for the extraction of pectin includes direct boiling, microwave heating (Garna *et al*., 2007; El-Nawawi and Shehata, 1987). Nevertheless, none of the methods extract all the pectin without causing some degradation. Microwave heating extraction takes no more than fifteen minutes to extract a satisfactory amount of pectin. However, its commercial application may not be realistic. Production of pectin by enzymes may take up to two days (Donaghy and McKay, 1994). The yield of pectin depends on extraction operation conditions such as temperature, extraction time, pH and the types of extraction peels (Fishman *et al*., 1999; Liu *et al*., 2006). The usual extraction procedure involves the use of water acidified to a pH between 1 and 4.5 with mineral acid, such as hydrochloric acid, sulphuric acid or nitric acid. The crushed citrus peels are added to this

acidified water and then slightly stirred at a temperature between 60-95°C for time period ranging from 30 min to several hours. The solid water mixture is subsequently separated by centrifugation or filtration. In the filtrate, the pectin is precipitated with the addition of alcohol. The filtrate is concentrated through evaporation in order to minimize the amount of alcohol used. The isolated pectin is then washed again with alcohol, dried and ground (khan *et al*., 2015). Citrus processing industry generates large amount of peel. Major gains for the industry could be the use of the waste to produce value added product, boosting indigenous production of high grade pectin as import substitute and the use of depectinized byproduct in cattle feed. The present study was therefore, undertaken to develop a simple and economical method for pectin extraction (khan *et al*., 2015).

2.5 Commercial pectins

Commercial pectins are structurally less complex due to the industrial extraction and purification process, which remove most of the neutral sugars (Schols and Voragen, 2002). Commercial pectins consist mainly of a backbone of α (1→4)-D-galacturonic acid with partial methyl esterification of the carboxyl groups (May 1990). At least 65% of the extracted material must be galacturonic acid, in order for extracted material to be classified as commercial pectin (Committee on Food Chemicals Codex, 1996; FAO, 2009).

2.5.1 Commercial processing of pectin

Even though most plant tissues contain pectin, the major sources for the commercial production of pectin are citrus peel and apple pomace. This is mainly because of the availability of citrus peel and apple pomace from commercial processing operations. In addition, both sources have high contents of galacturonic acid and good gelling properties for food applications. Pectin is usually isolated from either wet or dry fruit residues. Pectin, with better quality characteristics, is obtained from wet fruit residues, since pectin is a heat labile material and quality is lost in pomace drying process. However, apple pomace and citrus peel, in the wet state, are very perishable, especially due to microbial activity. Molds infestations could produce pectin degrading enzymes (i.e., pectinases), which render pectin in the raw material unacceptable for food uses. Therefore, in order to allow the storage, transportation, and supply throughout the year the starting material is generally dried (Rolin, 2002).

Pectin production is sometimes considered an art rather than a science due to the multiple factors influencing the extraction process and their inter-dependencies. Extraction temperature, extraction pH, and hot acid extraction time are considered the most important factors in the pectin extraction process because they have shown significant effects on different pectin characteristics; *e.g.* molecular weight, degree of esterification, and yield (Garna *et al*. 2007; Kalapathy and Proctor, 2001;Yapo *et al*., 2007). Generally, higher yields of pectin have been obtained at high extraction temperature, low extraction pH, and long hot acid extraction time. However, under these conditions the degradation of the pectin molecule is promoted, resulting in low molecular weights and low degree of esterification, which negatively affect the functional properties of pectin. Selecting the ideal combination of raw material and extraction conditions is critical in obtaining high yields of pectin, without compromising the desired quality (Miceli-Garcia, 2014).

The aims of this study were; (1) to investigate the effect of two extraction factors (*i.e.*, extraction temperature, and extraction pH) on pectin yield and (2) to establish optimum process conditions for pectin extraction from lemon peel.

2.6 Extraction procedures

The most coomonly used method for extractoin of pectin includes direct boiling and microwave heating. Direct boiling is a conventional method of pectin extraction, which takes approximately two hours to obtain a good yield of peictn. Due to a relatvely long period of direct heating, the exteracted pectin undergoes thermal degradation. Micro wave heating exteraction, on the other hand, takes no more than fifteen minutes to extract a satisfactory amount of pectin. Methods employing microwave heating are generally more effective in term of pectin yield and give better quality products as well. The yield of pectin also depends on the types of exteraction solvents used and of extera chelating agents such as EDTA and CDTA which helps in releasing pectin from cell wall (Srivistava and Malviya, 2015).

2.6.1 Water based extraction

The conventional water based extraction involves extracting the pectin using acidified water (pH up to 2) at temperature not more than 70 °C. The assembly is run for 2-4 h duration and pectic substances are precipitated using ethanol or isopropyl alcohol (Srivistava and Malviya, 2015).

A long list of various agents has been reported for the extraction of pectin from plant tissues. Extraction with the hot water is the simplest and oldest method for removing the pectic substances. The most commonly used acidifying materials are 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 have been also used. Meyer's and Rouse reported the use of different types of zeolites for extraction of pectin. A very low yield of pectin obtained from dreid orange peel was reported using Zeocarb as extractant at 85-90 °C. Double extraction at 85-88 °C for one hour using a cationic resin for the extraction of pectin from apple pomace has been reported to give higher yields and better gel strength of the product. IER has been extensively used in the purification and fractionalization of pectin.

Commercially, pectin is exteracted by treating the raw material with hot dilute mineral acid at pH about two. The precise length of exterction time varies with raw material, the type of pectin desired, and from one manufacturer to another. The hot pectin extract is separated from the solid residue as efficiently as possible. This is not easy since the solids are by now soft and the liquid phase are viscous. The viscosity increases with pectin concentration and molecular weight.

There is a compromise between efficeint extraction and solids separation and operating cost.the pectin extract may be further clarified by filteration through a filter aid. The clarified extract is then concenterated under vacuum. Powdered pectin can be produced by mixing the concenterated liquid from either apple or citrus with an alcohol(usually isopropanol). The pectin is separated as a stringy gelatnous mass, which is pressed and washed to remove the mother liquor, dreid and grounded (Srivistava and Malviya, 2015).

2.6.2 Microwave heating extraction

Compared with water based extraction microwave heating extraction reduces the extraction period considerably. A fifteen minute microwave heating period is enough to extract almost the same amount of pectin as that obtained from water based extraction with a three hour extraction period (Srivistava and Malviya, 2015).

During microwave heating considerable pressure builds up inside a material. The high pressure then modifies the physical properties of material tissues, breaking down the cell structure and improving the capillry porous structure of tissues. This feature allows better peneteration of

extracting solvent into the tissues; improving the subsequent extraction of pectin. Microwave extarction also gave a higher rate and amount of extraction than the simple water based methods.

Extraction of pectin from raw papaya(Carica Papaya Linn) pell citrus peel acerola, apple pomace, etc. has been reported using water based extraction procedure to procure pectic substances on large scale in industries. Pectin extraction from pumpkin with the aid of microbial enzymes, has also been a remarkable embankment for future and further scope of study in present field.

In the recent years supercritical fluid extraction technique, for extraction of natural polymers has come into existance. The relevancy of using the above technique for pectin extraction is yet to be proved over the other techniques available (Srivistava and Malviya, 2015).

2.7 Commercial pectins: extraction and manufacture

2.7.1Industrial sources

Pectin extraction at the laboratory level and for research purposes generally involves mild conditions and more complex steps than those found at the industrial level. The more severe extraction processes used at the industrial level are generally more detrimental to the pectin molecules with regard to substituents (neutral sugar side chains, esterified groups) and molecular mass.

Apple pomace and citrus peel are the two raw materials traditionally used for industrial extraction of pectins. Both materials contain high amounts of pectic substances and are available in abundant supply as residues from juice production. However, they produce slightly different pectins, which make one or the other more suitable for specific applications, although both have well-recognized desirable and commercially attractive properties. Dried apple pomace generally contains 15 to 20% pectin, whereas dried citrus peel generally yields around 30 to 35% of pectin.

Before industrial extraction, the raw materials are generally subjected to a pre-treatment (blanching, washing, drying, etc.) to inactivate enzymes that otherwise would rapidly degrade the pectin molecules, and to increase the product stability during transportation and storage.

Extraction of pectins from the raw material is usually performed by acid treatment (pH 1.5 to 3) at high temperature (50 to 90 °C), using hydrochloric acid, and nitric acid or, in some cases, sulfuric acid. This step enables extraction and solubilization of pectin materials from plant tissues. Under these conditions, degradation reactions such as de-esterification and depolymerization will occur. Therefore, the extraction conditions, namely temperature, time, and pH, should be carefully controlled to achieve the desired final properties of the product. The pectin raw extract is then separated from the peel or pomace residue by filtration or centrifugation process. The pectin is then separated from the purified extract by precipitation with alcohol (isopropanol, ethanol, or methanol) or by precipitation with an insoluble salt by addition of a polyvalent cation, usually aluminum. The precipitate obtained is washed with alcohol and pressed to remove soluble impurities, and finally dried and milled to yield powdered pectin. The pectins thus obtained have typically a DE higher than 50% and are known as HM pectins; de-esterification is also performed at the industrial level to yield pectins with lower DE (<50%) known as LM pectins (Stephen, *et al*., 2006).

2.8 Genral properties of pectin

Pectin is soluble in pure water. Monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water; di-and trivalent cations salts are weakly soluble or insoluble. Dry powdered pectin, when added to water, has a tendency to hydrate very rapidly, froming clumps.These clumps consist of semi dry packets of pectin contained in an envelope of highly hydrated outer coating. Further solubilization of such clumps is very slow. Clumps formation can be prevented by dry mixing pectin powder with water-soluble carrier material or by the use of pectin having improved dispensability through special treatment during manufacturing. Dilute pectin solutions are Newtonian but at a moderate concentration, they exibit the non-Newtonian, pseudo plastic behaviour characteristics. As with solubility, the viscosity of a pectin solution is related to the molecular weight, degree of esterification, concentration of the preparation, and the pH and presence of counter ions in the solution. Viscosity, solubilty, and gelation are generally related. For example, factors that incraeses gel strength will increase the tendency to gel, decrease solubility, and increase viscosity, and vice versa (Srivistava and Malviya, 2015). The native pectic polysaccharides (protopectins) present in plant cell walls show complex structural characteristics, including a diversity of neutral sugars that bond to the main galacturonate

backbone and varied degrees of branching. However, commercial pectins are typically less complex structurally due to the structural changes and breakdown taking place during the industrial extraction processes.

Pectins in an aqueous environment are not stable molecules; depending on the pH and temperature, pectin molecules may undergo several chemical reactions and modifications. Under acidic conditions, glycosidic bonds and methyl-ester linkages may undergo hydrolysis to different extents. Hydrolysis of the more sensitive glycosidic linkages, like those involving the neutral sugar side chains, may lead to the increase of the galacturonic content and decrease of the neutral sugar content of the acid-treated pectins. During acid treatment at reduced temperature, the rate of glycosidic-bond hydrolysis is much slower than the rate of de esterification; therefore, preparations of low methoxyl pectins may be obtained by acid treatment without extensive main-chain breakdown and decreasing of the pectin molecular weight. Any increase of temperature increases the rate of the b-elimination reaction more than that of the de-esterification (khan *et al*., 2015).

Under alkaline conditions, or even at a pH close to 7, especially at elevated temperatures, extensive and rapid degradation may occur, involving both de-esterification and cleavage of glycosidic bonds, the last occurring generally by a trans-b-elimination mechanism. The degradation of pectins under these conditions increases with temperature and with the degree of methyl-esterification (DE). Kinetic considerations about both the de-esterification and depolymerization processes have been reported, including values for the rate constants and activation energies. This low stability of the pectin molecules makes them less attractive for use in heat-processed foods of low acidity.

Pectins are macromolecules that contain a large number of ionizable carboxylic groups; therefore, they are anionic polyelectrolytes, having a strong affinity for small counter-ions. Most of the conformational and functional properties of pectins depend on their polyelectrolytic properties (Stephen *et al*., 2006).

With respect to the physical properties of pectin molecules in aqueous media, it is not surprising to observe that they depend on their chemical characteristics, including their structure and size, and also on the solvent properties (pH, ionic strength, presence of co-solutes). Water is a good

solvent for pectins. Aqueous solutions up to about 4% (w/w) may be prepared, but with expected limitations with regard to the viscosity and dispersion difficulties. Solubility in water decreases as the DE decreases. The decreased solubility observed for low-methoxyl pectins may be overcome by converting the free carboxyl groups to more soluble salt forms (e.g., sodium or potassium salts). Generally, pectins are insoluble in organic solvents. Their precipitation from aqueous solutions by the addition of watermiscible organic solvents such as ethanol, isopropanol or methanol, or by addition of polyvalent cations (e.g., copper, quaternary ammonium), is often used in recovery and purification procedures both at the industrial and laboratory level. Pectins are commonly used as a gelling and thickening agent in foods. As for solubility, the viscosity of pectin solutions is also dependent on structure, DE, molecular weight, pectin concentration, solvent properties, and temperature. High methoxyl pectin (50% DE) forms gels under acidic conditions in aqueous media of high sugar content, whereas low methoxyl pectin forms gels in the presence of calcium ions (Stephen *et al*., 2006).

2.9 Functionalities and applications of pectin

Pectin is commonly utilized as a gel forming agent in food and pharmaceutical applications (Liu *et al*., 2003). The gel forming ability of pectin depends primarily on the degree of esterification (DE), which is the proportion of galacturonic acid units that are esterified with methanol (Sriamornsak 2003). Commercial pectins are classified based on their DE; high methoxyl pectins (HM, DE>50) and low methoxyl pectins (LM, DE <50). A pectin gel is formed when portions of the homogalacturonan backbone are crossed-linked forming a continuous three-dimensional network, which is able to entrap water and solutes (Willats *et al*., 2006). The "joints" of homogalacturonan portions are often referred as "junction zones". Various forces may be involved in the formation of a junction zone (Thakur *et al*., 1997). In high methoxyl (HM) pectins junction zones are formed by hydrogen bonds and hydrophobic forces between methoxyl groups of pectin 13 molecules. To permit the formation of junction zones in HM pectins, low pH (<3.6) and soluble solids (usually sucrose) at a concentration higher than 55% (w/w) are required. In contrast, LM pectins require the presence of divalent metallic cations, such as calcium, to form junction zones by ionic cross-linking between non-esterified carboxyl groups (Willats *et al*., 2006). The rheology of pectin gels depends on properties of the pectin, such as molecular weight, attached neutral sugars, and degree of acetylation (Baïer *et al*., 1994).

Pectin, like most naturally occurring polysaccharides, can create viscous solutions; viscosity depends largely on the concentration of pectin and its molecular weight. At low concentrations (<0.5% w/w), pectin solutions exhibit Newtonian behavior, but as the concentration increases pectin solutions exhibit non-Newtonian, pseudoplastic characteristics. Pectins with higher molecular weight will exert higher viscosity. Other factors, such as degree of esterification, solvent, pH, soluble solids, and temperature also influence the viscosity of pectin (Voragen *et al*., 1995).

In addition to the gelling and thickening properties, certain pectins possess emulsifying and/or emulsion stabilizing properties. Studies have suggested that the emulsifying property of pectin could be originating from (a) the presence of acetyl groups, which enhance the hydrophobicity of pectin, (b) the protein residues present within the pectin, or (c) a combination of both (Leroux *et al*., 2003). Studies by Endress and Rentschler (1999) suggested that the emulsion stabilizing property of citrus and apple pectin were mainly based on the increase of viscosity of the emulsion, suggesting that pectins with a high degree of esterification were effective in stabilizing oil-in-water emulsions (Miceli-Garcia, 2014).

In addition, pectin is able to stabilize proteins in suspension. For example, pectin can stabilize milk protein under acidic conditions. It is thought that the homogalacturonan (HG) region of pectin, which is negatively charged, bind to the positively charged proteins in milk, preventing aggregation and sedimentation of casein (Willats *et al*., 2006).

2.9.1 Pharmaceutical applications of pectin

The use of pectin in the pharmaceutical industry is growing, but the amount used is much smaller compared to the food uses. Pectin is used in many pharmaceutical preparations as an excipient, thickener, stabilizer, film coating, and binding agent. Due to its ability to bind positively charged heavy metal ions, pectin is used as a detoxifying agent. It has been used to remove toxic heavy metal ions, such as lead and mercury, from the gastrointestinal tract and respiratory organs of individuals who have been poisoned with heavy metals. Since pectin is able to delay blood clotting, it is also used as hemostatic device to control hemorrhage or localized bleeding (Sriamornsak, 2003).

Pectin has been widely utilized as a drug carrier, and as ingredient in controlled and sustained drug release formulations (Wong *et al*., 2011). Delivery systems designed to release drugs in the colon need to protect the drug during transit through the stomach and small intestine. The resistance of pectin to digestion in the upper gastrointestinal tract has allowed its use in the development of colon-specific drug delivery systems (Liu *et al*., 2003). Tablets coated with a pectin film as well as pellets and micro particles made from pectin-based matrices are commonly used to successfully deliver drugs to the colon (Wakerly *et al*., 1996; Wong *et al*., 2011).

2.9.1.1 Cholesterol regulation

The mechanism of the possible hypocholesterolemic activity of pectin is not well understood. It appears that the viscosity of pectin is related to its possible hypocholesterolemic activity. Pectin preparations with high viscosity appear to be more effective in lowering cholesterol than are pectin preparations with lower viscosity. Highviscosity pectin is thought to lower cholesterol levels by raising the excretion of fecal bile acids and neutral sterols. High-viscosity pectin may interfere with the formation of micelles and/or lower the diffusion rate of bile acid and cholesterol-containing micelles through the bolus, consequently diminishing the uptake of cholesterol and bile acids. Numerous studies have demonstrated that pectin has favourable effects on lipids. In a small early study, administration of 15 grams of pectin daily for three weeks resulted in a mean 13% reduction in plasma cholesterol levels. There was no effect on plasma triglyceride concentrations. Subsequently, giving 40 to 50 grams of pectin daily significantly lowered cholesterol levels in both normolipidemic and hyperlipidemic subjects. In another study, a pectin-supplemented diet (without other dietary or lifestyle changes), significantly reduced plasma cholesterol in volunteers evaluated to be at medium to high risk for coronary heart disease due to hypercholesterolemia. This was a double-blind, placebo-controlled trial. Treatment continued for 16 weeks. The pectin was credited with decreasing plasma cholesterol 7.6% and LDL cholesterol 10.8% (Sharma *et al*., 2006)

2.9.1.2 Anti-cancer action

Lemons, oranges and grapefruits are pectin rich fruits that may help decrease cancer tumor formation. According to Memorial Sloan-Kattering Cancer Centre, the citrus pectin acts as a ligand for galectin-3, a protein involved in cell growth and cell cycles. Elevated galectin-3 is

associated with inflammation of the heart and cancer tumors. Institute, N.B. and Walding had reported that pectin supplementation in the diet forbids excess galectin-3 from binding to receptors that might result in the spread of cancer cells through angiogenesis or blood vessel growth.

It has also been used as a fat substitute in spreads, ice-cream and salad dressings. (Liu *et al*., 2003) reported that in terms of nutrition, pectin has been shown to lower blood cholesterol levels and low-density lipoprotein cholesterol fractions, which is beneficial for human health (Shaha *et al*., 2013).

Modified citrus pectin, when administered orally to rats, was found to inhibit spontaneous prostate carcinoma metastasis. It had no effect on the growth of the primary tumour. Injected modified citrus pectin was found to inhibit metastasis of melanoma cells in mice. The mechanism of these anticarcinogenic effects is not clear.

Galectins comprise a family of galactoside-binding mammalian lectins. Lectins themselves comprise a group of hemagglutinating proteins found in plant seeds, which bind the branching carbohydrate molecules of glycoproteins and glycolipids on cell surfaces, resulting in agglutination or proliferation, among other things. Galectins are proteins that can bind to carbohydrates via carbohydrate recognition domains (CRDs). At present, the galactin family includes 10 members. Apparently, galectins are secreted from cells via nonclassical secretory pathways. Galectin-3, one of the members of the family, is thought to be involved in mitosis and proliferation. On the cell surface, galectin-3 mediates cell-cell adhesion and cell-matrix interaction via binding to its complementary glycoconjugates, such as laminin and fibronectin, and thereby is thought to play an important role in the pathogenesis of cancer metastasis.

Some metastic events may involve cellular interactions that are mediated by cell surface components, including galectins. The galactose-containing carbohydrate side chains of modified citrus pectin may interfere with these cellular interactions by competing with the natural ligands of the galectins and by doing so, inhibit the metastatic process. It is thought that galectins may play a role in human prostate cancer, and in particular, human prostate cancer metastasis (Sharma *et al*., 2006).

2.9.2 Food applications of pectin

Pectin is known for being the traditional gelling agent in jams and jellies (May, 1990). Nowadays there is a large variety of commercial pectins, differing mainly in their degrees of esterification, available to meet the requirements of different jams or similar fruit containing, sugar rich, highly viscous systems (Voragen *et al*., 1995). Although the use of pectin in jams and jellies is still one of the largest markets for pectin (Brejnholt, 2010), it is utilized in other foods as a thickener, texturizer, emulsifier, and stabilizer (FAO, 2009)

Due to its stabilizing and thickening properties, pectin is widely used in the dairy industry. To prevent aggregation and precipitation of caseins, pectin is used in low acid (pH 3.5 - 4.2) milk products that are heat processed (Voragen *et al*., 1995). Pectin has various roles in yogurt depending on the type of product; as a thickener in spoonable yogurts; as a water binder in stirred yogurts; and as emulsifier and to provide fat-like mouthfeel in low-fat yogurts. In addition, pectin is used in the production of fruit bases for yogurts to ensure uniform distribution of the fruit and to reduce color migration from the fruit to the yogurt (May, 1990; Voragen *et al*., 1995).

Pectin has a variety of applications in the bakery industry. It is used to retain moisture and to improve volume, flexibility, and softness in breads. In frozen dough, pectin is used to delay the retrogradation of starch, while stabilizing the volume of the dough during freezing. The stabilizing and thickening properties of pectin have allowed its use in many other food products, such as mayonnaise, salad dressing, tomato ketchup, protein foams, and beverages (Pilnik and Voragen, 1992).

New applications for pectin have emerged due to the increasing awareness of healthy life-styles of consumers, along with the increasing demand for functional food products. For example, pectin is commonly used in low-fat foods as a fat replacer (Min *et al*., 2010). Foods can be also coated with pectin and polyvalent cations prior to frying, to reduce the absorption of oil (Gerrish and Carosino, 2001).

2.10 Lime overview and varieties

Citrus trees are among the favorite fruit trees grown around world. They are relatively small and widely adapted. The main types grown are lemon, lime, orange, mandarin (tangerine), tangelo, grapefruit, and pummelo. Some culinary and ornamental citrus species are also grown (Chang and Bay- Peterson, 2003).

Limes, along with lemons and citrons, are a species in the common-acid fruit group. Common-acid fruits have high citric-acid content and an elliptical shape with a nipple end, distinguishing them from other species within the Citrus genus. These fruits are believed to have originated in the Northeast India, Indo-China region, and followed westward trade routes to the Mediterranean and onward to the Americas. Limes are highly freeze-intolerant and require long periods of heat to suitably mature, so production is limited to areas with mild to warm winters (subtropical to tropical). Like most citrus crops, limes are ever bearing and harvest typically occurs multiple times throughout the year, with mature fruit occurring in January-February, June-July, and September-October.

There are three major commercially-produced lime varieties: the Persian lime, the key lime, and the makrut lime (table 2.1). The Persian and/or Tahitian lime is the most widely produced lime globally with Mexico being the largest producer. The fruit is medium-sized, with an oval-oblong shape (resembling a small lemon), smooth skin, thin rind, juicy and very acidic with "true" lime flavor. The Persian lime has lower heat requirements for fruit to reach maturity and size preference, and is also slightly more cold/freeze tolerant. This lime is preferred by Americans, due to large size and high juice content.

The key lime, also known as the West Indian lime or Mexican lime, is produced heavily in India and parts of Mexico, where it is the preferred variety for domestic use. It is very smooth-skinned with a thin rind, round, juicy, highly acidic with a strong aroma (Reuther, *et al*., 1967).

Table 2.1Major lime varieties and description

Unit type	Other name	Appearance
Key lime (citrus x aurantiifolia)	West Indian, bartender's, Omani or Mexican	1-2 in diameter, highly acidity, strong aroma, tart and bitter

		7-8 percent citric acid
Persian lime (citrus x latifolia)	Shiraz limoo, Tahitian,bearss (seedless)	2-5 in diameter, slight nippled end,ripens to yellow but sold green
Makrut lime (citrus hystrix)	Kaffir	2 in diameter, rough-bumpy skin, thick rind Aromatic leaves used in cooking

Source: Reuther, *et al*., 1967.

2.10.1 Lemons

Lemons, scientifically known as *Citrus limon.* Lemons are oval in shape and feature a yellow, texturized outer peel. Like other citrus fruits, their inner flesh is encased in segments, with the average lemon having eight to ten.

While most lemons are tart, acidic and astringent, they are also surprisingly refreshing. The two main types of sour lemons are the Eureka and the Lisbon. The Eureka generally has more texturized skin, a short neck at one end and a few seeds, while the Lisbon has smoother skin, no neck and is generally seedless. In addition to these sour lemons, there are also some varieties that are sweet in flavor. One notable example is the Meyer lemon that is becoming more popular in both markets and restaurants.

Lisbon (29% of lemon plantings). - is very similar to Eureka, although it is generally considered that Lisbon is thornier and produces its main crop in winter. It tends to be more cold tolerant and produces more fruit on the inside, wind-protected parts of the canopy. This may result in better quality skins (less wind rub) even if boundary windbreaks have been established. Lisbon is vigorous, thorny, and densely foliaged with a more upright growth habit. Fruit tend to be produced on the inside of trees and the main crop is produced in winter. Lisbon is the most resistant variety to adverse climatic conditions (heat and cold).

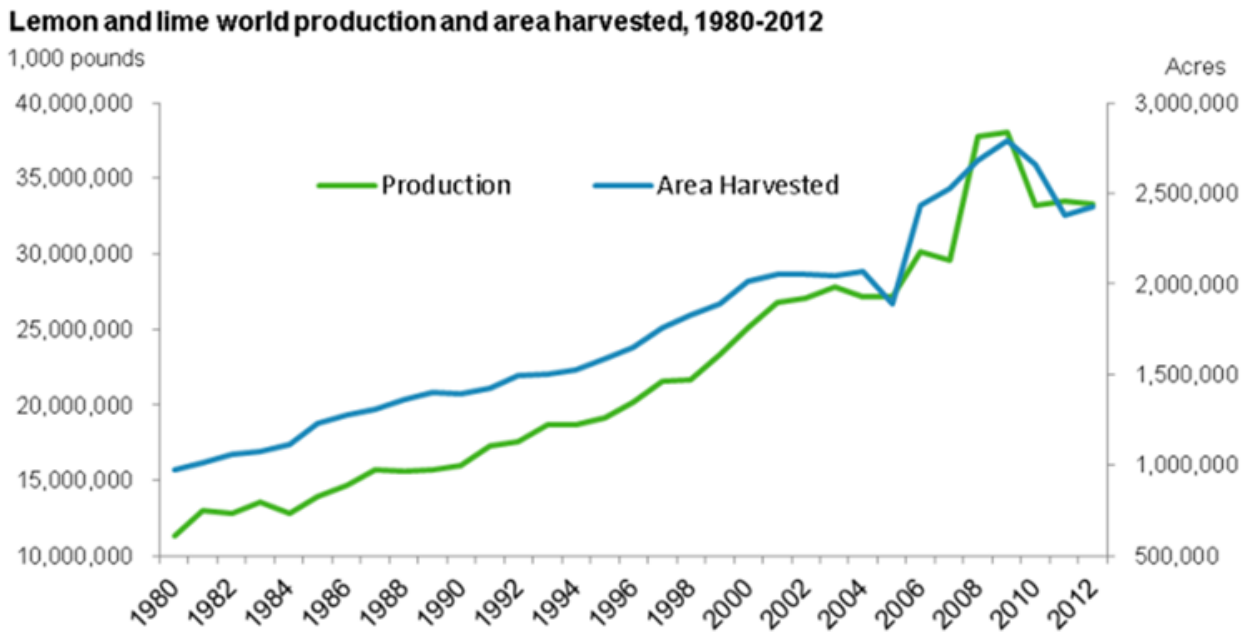
Lisbon lemon is of Portuguese origin and was first grown in Australia in 1824. Lisbon lemon is also popular in California, Arizona, Uruguay and Argentina. The selection currently available (Prior nucellar) was imported into Australia from California in 1965. Another selection known as

Limoniera 8A was imported into Australia and released from plant quarantine in 2000. Limoniera 8A is currently the most popular Lisbon lemon selection in California and Arizona. Lisbon is a winter producing lemon, with production of one main crop in winter and early spring.

Figure 2-4 Picture which shows Lisbon variety (left) and Mexican lime on the (right)

2.10.2 Global Lime Production

World lemon and lime production has been increasing annually since 1980, with world production reaching 33.3 billion pounds in 2012, up almost threefold from 1980's production of 11.3 billion pounds (Fig. 2.5). For lemons, Argentina, Turkey, the United States, Spain, Italy and South Africa are the largest producers as of 2012 (U.S. ITC, 2013). For limes, Mexico and Brazil are the world's largest producers (United Nations, Food and Agriculture Organization (UN/FAO), 2003). Both countries produce large quantities of key limes, with Mexico producing Persian limes as well. However, constrained by the lack of refrigeration, key limes are the preferred lime in Mexico due to its longer shelf life (Spreen, 2000). Many countries produce limes but consumption tends to remain in their respective domestic markets with little product exported. sosdddd



Source: United Nations, Food and Agriculture Organization, FAOSTAT, 2013.

Figure 2-5 Shows lemon and lime world production and area harvested

India is the largest, averaging 609,196 acres, during 2010-12 (about 25 percent of global acreage), followed by Mexico with 637,012 acres (15 percent of global acreage) and China with 292,312 acres (12 percent of global acreage). Brazil and Argentina account for 5 percent and 4 percent of global harvested acreage, respectively, rounding out the top five. The remaining 40 percent of harvested acreage is spread among 103 other countries (UN/FAO, 2014).

With respect to combined global lemon and lime production, China accounted for roughly 17 percent of 2009-12 total average volume, India with 15 percent, and Mexico 13 percent (UN/FAO, 2014). For the same period, the United States came in at just 5 percent of total lemon and lime production. Most countries that are capable will produce small amounts of lemons or limes to meet domestic demand. Only three countries account for more than half of the world's lemon and lime harvested acreage (Fig. 2.5).

2.11 Consumption of Pectin in Ethiopia

Ethiopia has been importing pectin for its uses in food processing and pharmaceutical industries with a hard currency from abroad. According to the data obtained from Ethiopian Customs Agency (2014), the annual use of pectin in food, beverage, drug and pharmaceutical sectors is limited and almost constant over the last five years (Table 2.2). One of the basic reason is

Ethiopia has been importing finished products in which pectin has been added as an ingredient. Inland production and utilization of pectin will reduce the import volume of finished products for which pectin is required, and also reduce the import volume of pectin for the various sectors.

Table 2.2 Pectin imported to Ethiopia in the year 2010-2014

Year	Import volume (kg)	Value, Birr
2010	4,962.00	1,570,350.12
2011	6598.00	1,880,430.00
2012	6,842.00	2,011,548.00
2013	7,102.00	2,059,580.00
2014	7,176	2,088,216.00

Source: (CSA, 2014)

The future demand for processed foods in general is mainly a function of urbanization, income, price and change in the consumption habits of the population. As income rises and urbanization progresses, there will be a shift towards more expensive but conveniently packed and available foods. Urban population is growing by more than 4% per annum in Ethiopia. Moreover, users of jam and marmalade like pastries are growing very rapidly currently in the country. Pectin is the main ingredient in the production of jams and marmalades. The import and domestic production of jams and marmalades is around 723 ton. The amount of pectin required to make jams and marmalades varies from 3.3-5%. From this figure we can roughly estimate the total amount of pectin that is being consumed in our country and its around 30 ton.

3. MATERIALS AND METHODS

The experiments were carried out at Addis Ababa Institute of Technology in the School of Chemical and Bio-engineering laboratory. FTIR spectroscopy result was analyzed in the Department of Chemistry at the College of Natural Science.

3.1 Materials

3.1.2 Chemicals and reagents used

The following analytical grade chemicals were used for extraction of pectin. These include nitric acid (HNO_3), phenol red indicator, hydrochloric acid (HCl), sodium hydroxide (NaOH), distilled water, sodium chloride (NaCl) and ethanol. All the chemicals and reagents were purchased from chemical stores in Addis Ababa.

3.2 Equipment used

The major equipment required for extraction of pectin from lemon were centrifugal mill, water bath, thermometer, measuring cylinder, round bottomed flask, funnel, micro pipette, beakers (20ml- 1000ml), centrifuge, oven and filter paper

3.3 Raw material Preparation

Seven kilograms of Lisbon and Mexican varieties were purchased from Upper Awash Agro-Industry Enterprise. The samples were washed carefully with tap water to remove dirt soil from surface; they were cut into slices (2-3 mm thickness) with a sharp knife. The juice was extracted by juice extractor. After juice extraction the peel was chopped into small pieces in a vegetable cutter dried at 60 °C for 48 h in an oven followed by grinding into powder using centrifugal mill. The powder was then sieved using sieve and packed in low density polyethylene bags. The obtained powder was finally stored at ambient temperature for further use.

3.4 Extraction

Method for extraction of pectin consisted of adding 150 ml of distilled water to 5g pectin peel powder in a beaker and addition of nitric acid adjusted at different pH(1,2.5,and 4) and then heating the mixture to 40 °C, 60 °C and 80 °C for 2 h, respectively, in a hot water bath. The hot acid extract was filtered using centrifugal machine at 3000 rpm. The pectin extract obtained from

different extractions, after cooling to room temperature, was precipitated with 96% ethanol (alcoholic precipitation). The filtrate was coagulated using an equal volume of 96% ethanol and left for 2 h to allow the pectin to float on the surface. The gelatinous pectin flocculants were then skimmed off. Filtration was again done for separation of coagulated pectin. The coagulated pectin was then kept for drying in oven at 50 °C for 24 h. After drying the pectin was ground. The dried pectin obtained from the two varieties' was packed in food grade polythene bags and stored in a cool dry place until further analysis. Finally percentage yield was calculated (Altaf *et al*., 2015).

3.5 Characterization of pectin

Characterization (physicochemical properties) of pectin extract:

The moisture content and ash content of pectin was determined by (AOAC, 2000) method. Equivalent weight, methoxyl content, anhydrouronic acid and degree of esterification were determined by the methods described by Ranganna (1995).

3.5.1 Percentage yield

Yield % of pectin is based on the gram of peel sample taken, and was calculated by formula as given below

$$\text{Ypec (\%)} = 100 \text{ x } (\frac{P}{B_i}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

Where Ypec (%) is the extracted pectin yield in percent (%), P is the amount of dry pectin in g and B_i is the initial amount of lemon peel in gram.

3.5.2 Ash content

A dry porcelain dish containing about 2g sample was placed in a muffle furnace (GALLENKAMP, Model FSL 340-0100, U.K.) set at 550 °C for 1h and was cooled in a desiccator and then weighed. The ash content was determined according to AOAC (2000) by applying the formula:

$$\text{Ash \%} = \frac{(W_2 - W)}{(W_1 - W)} x100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

Where:

W = is weight in grams of empty dish

W_1= Weight in grams of the dish plus the dried test material

W_2 = is Final weight of dish with Ash.

3.5.3 Moisture content

Moisture of the pectin was determined according to AOAC (2000). A clean dried dish was weighed, and 5g of the sample was transferred to the dish. The dish was then placed in the oven (Memmert 854 Schwabach, West Germany) at 102 °C for 5h and cooled in desiccators and re-weighed. Then, the moisture content was estimated by the formula:-

$$\text{Moisture content \%} = \frac{W_2 - W_3}{W_2 - W_1} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots... (3.3)$$

Where:

W_1 = weight of crucible (g)

W_2 = weight of fresh sample and crucible (g)

W_3 = weight of dry sample and crucible (g)

3.5.4 Determination of equivalent weight

Equivalent weight was determined using Ranganna’s method (1995). 0.5 g sample was taken in a 250 ml conical flask and 5 ml ethanol was added. 1 g of sodium chloride and 100 ml of distilled water was then added. Finally 6 drops of phenol red was added and titrated against 0.1 N NaOH. Titration point was indicated by purple color. This neutralized solution was then stored for determination of methoxyl content. Equivalent weight will be calculated by the following formula：

$$\text{Equivalent weight} = \frac{weight\ of\ sample(g) * 100}{ml\ of\ alkali * Normality\ of\ alkali} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

3.5.6 Methoxyl content

Determination of MeO content was carried out using the Ranganna’s method (1995). The neutral solution was collected from the determination of equivalent weight, and 25 ml of sodium hydroxide (0.25 N). The mixed solution was stirred thoroughly and kept at room temperature for

30 min. After 30 min, 25 ml of 0.25 N hydrochloric acid was added and titrated against 0.1N NaOH to the same end point as before like in equivalent weight titration. The methoxyl content or degree of esterification is an important factor in controlling the setting time of pectin, the sensitivity to polyvalent conations, and their usefulness in the preparation of low solid gels, fibers and film. Methoxyl content was determined by saponification of the pectin and titration of the liberated carboxyl group.

$$\text{Methoxyl content \%} = \frac{ml\ of\ alkali * Normality\ of\ alkali * 3.1}{weight\ of\ sample(g)} \quad \ldots\ldots\ldots \quad (3.5)$$

3.5.7 Determination of total anhydrouronic acid content (AUA)

Total AUA of pectin was obtained using the formula recommended by Mohamed & Hasan (1995).

$$\text{\% of AUA} = \frac{176 * 0.1z * 100}{w * 1000} + \frac{176 * 0.1Y * 100}{w * 1000} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.6)$$

When molecular unit of AUA (1 unit) = 176 g

Where,

z = ml of NaOH from equivalent weight determination.

y = ml of NaOH from methoxyl content determination.

w = weight of sample, g

3.5.8 Determination of degree of esterification (DE)

The DE of pectin was measured on the basis of methoxyl and AUA content (Owens et al., 1952) and calculated by flowing formula.

$$\text{\% DE} = \frac{176 * \%MeO}{31 * \%AUA} \text{x}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.7)$$

3.6 Experimental design and data analysis

The experimental design selected for this study was general factorial design and the response variable was the pectin yield gained. Design-Expert 7.0 software was used to determine the effect of the two operating variables on the percentage of extraction of pectin from the two citrus

varieties. These are extraction temperature and pH. Significance of the result is set from analysis of variance (ANOVA). The independent variables were extraction temperature, and pH, and these variables were selected to optimize the conditions for extraction of pectin. The data required to optimize the process conditions for pectin extraction was collected by conducting the experiments; followed by analysis using the Design-Expert software 7.0.0 resulting in suitable model equation.

3.6.1 Experimental design

In this research lemon and lime peel powder were subjected to the extraction of pectin using hot acid by varying the temperature and the pH to obtain different yield. Experimental data analysis was done using Design Expert 7.0.0 software. The experimental design selected for this study was three-level-two-factor general factorial design and the response variable measured was percent (%) yield. In addition analysis of physicochemical properties of pectin was done at School of Chemical and Bio Engineering laboratory, AAIT, and FTIR analysis was carried out at Chemistry Department, Faculty of Natural Science. The two independent variables studied for the extraction of pectin were extraction temperature, and pH. The independent variables interaction effect was analyzed to obtain maximum percentage yield.

General factorial with two factors and three levels was carried out for each variety; total of 27 experiments were conducted for the two varieties. The twenty seven experiments were done and the data was statistically analyzed using Design-Expert Software 7.0.0 to obtain a suitable model equation for the % yield as a function of the independent variables.

The usual extraction procedure involves the use of water acidified to a pH between 1 and 4.5 with mineral acid, such as hydrochloric acid, sulpheric acid or nitric acid at a temperature between 60-95°C for time period ranging from 30 min to several hours (Khan *et al*., 2015).

The conventional water based extraction involves extracting the pectin using acidified water (pH up to 2) at temperature not more than 70 °C. The assembly is run for 2-4 h duration and pectic substances are precipitated using ethanol or isopropyl alcohol (Srivistava and Malviya, 2015). The factors chosen for this experiment were selected according to literatures and for choice of extraction time priliminary experments were conducted.

Table (3.1) lists the range and levels of the two independent variables studied. The lower and higher levels are chosen by considering the operating limits of extraction of pectin and its processing conditions. The complete experimental design matrix for the general factorial design was shown and the order in which the runs were made was randomized to minimize systematic errors. Independent variables and levels used in for the extraction of pectin from lemon and lime peels are listed below in Table (3.2). The complete experimental design matrix for the general factorial design was shown and the order in which the runs were made was randomized to minimize systematic errors.

3.6.2 Design of experiment

The experiment started with selected extraction time of 2 h based on literatures of (Khan *et al*., 2015) and a series of preliminary tests. At pH=1 and at temperature 60 °C and extraction time of 60 min, 90 min, and for 120 min the preliminary tests were conducted and pectin yield of 16.43, 18.32, and 19.6 were found respectively. Khan *et al.* (2015) suggests with regard to pectin precipitation as a function of heating time, it can be said that 30 min to 1 h heating time is sufficient to isolate maximum percentage of pectin as on further increase in heating time did not result in any increase in pectin yield. Extraction time beyond 120 min is not economically feasible because heating at temperature 60 °C and above for that longer period of time at higher temperature could bring biological loss. Each treatment was conducted in triplicates and completely randomized design (CRD) was used in this study.

Table 1.1 Independent variables and levels used in general factorial for pectin extraction

Variables	Factors coding for Lisbon variety	Factor coding for Mexican variety	Unit	Level coded factors		
				(1 0)	(0 1)	(-1 -1)
Temperature	A	A	°C	40 °C	60 °C	80 °C
pH	B	B		1	2.5	4

Below in Table (3.2) the complete experimental design matrix of general factorial design was shown. The order in which the runs were made was randomized to minimize systematic errors.

Table 3.2 The complete experimental design matrix

Run	Coded factors		Actual factors		Response 1
	Factor 1 A. Temperature °C	Factor 2 B. pH	Temperature °C	pH	Yield %
1	(1 0)	(1 0)	40	1	
2	(1 0)	(1 0)	40	1	
3	(1 0)	(1 0)	40	1	
4	(0 1)	(1 0)	60	1	
5	(0 1)	(1 0)	60	1	
6	(0 1)	(1 0)	60	1	
7	(-1 -1)	(1 0)	80	1	
8	(-1 -1)	(1 0)	80	1	
9	(-1 -1)	(1 0)	80	1	
10	(1 0)	(0 1)	40	2.5	
11	(1 0)	(0 1)	40	2.5	
12	(1 0)	(0 1)	40	2.5	
13	(0 1)	(0 1)	60	2.5	
14	(0 1)	(0 1)	60	2.5	
15	(0 1)	(0 1)	60	2.5	
16	(-1 -1)	(0 1)	80	2.5	
17	(-1 -1)	(0 1)	80	2.5	
18	(-1 -1)	(0 1)	80	2.5	
19	(1 0)	(-1 -1)	40	4	
20	(1 0)	(-1 -1)	40	4	
21	(1 0)	(-1 -1)	40	4	
22	(0 1)	(-1 -1)	60	4	
23	(0 1)	(-1 -1)	60	4	
24	(0 1)	(-1 -1)	60	4	
25	(-1 -1)	(-1 -1)	80	4	
26	(-1 -1)	(-1 -1)	80	4	
27	(-1 -1)	(-1 -1)	80	4	

3.7 Experimental setup

Lemon and lime peels collected from upper awash agro- enterprise were washed with tap water to remove dirt. The juice in the lemon is extracted in juice extractor and the peels were dried in oven for 24 h. The dried peels were grinded in centrifugal mill and packed for analysis. Now it's the time to extract the pectin out of the powdered peels using water bath at temperature of 40 °C, 60 °C, and 80 °C. 150 ml of distilled water at different pH value was adjusted at (1, 2.5, and 4) and mixed with 5 gram of powder for two hours in 250 ml conical flask. After this the solution is separated using centrifugal machine which runs at 3000 rpm two separate the phases. At this time the precipitate is mixed with equal volume ratio of 96% ethanol. After addition of ethanol the pectinase substance comes to the top and for the second time we need to separate the gelling substance and dry it at 50 °C for 24 h. The product from the oven is grinded and packed for further analysis.

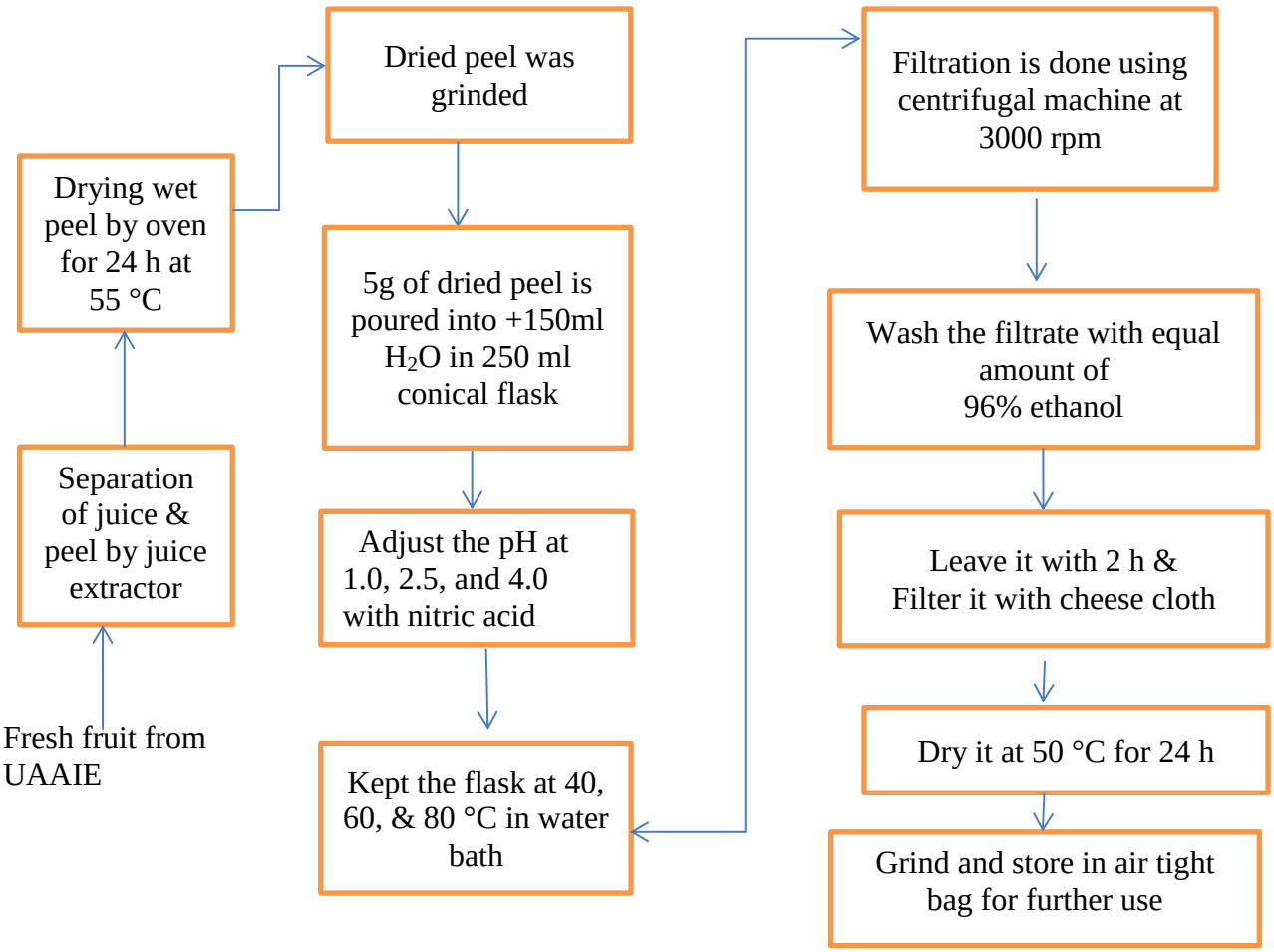


Figure 3-1 Schematic diagram of experimental setups of pectin production

3.8 Process description of pectin

The main raw material for pectin production in our case was dried peel of two varieties Mexican and Lisbon. Two varieties were purchased from upper-awash agro industry enterprise. The fruits were washed with warm water to remove any dirt and dust and then peeled. Because of its size the Mexican variety was peeled using juice extractor whereas Lisbon variety was peeled by manually. Peels were then cut into small pieces for efficient drying. The peels were then dried in an oven 50°C for 24 hours. The dried peels were then grinded using centrifugal mill to prepare fine powder with 70 MESH size for extraction of pectin

For pectin production in the laboratory 150ml of distilled water was added to 5g of each citrus peel powder followed by adjustment of pH to desired value of (1, 2.5, and 4) in 250 ml conical flask. Then the mixture was heated for each different pH medium with temperature of 40 °C, 60 °C, and 80 °C for each variety for 2 hours in hot water bath then the hot acid extract was filtered through muslin cloth.

For each variety, three different pH medium of extraction at three different ranges of extraction temperatures were carried out and collected separately for further experiments. The filtrate was cooled and the concentrated liquid is mixed with 96% ethanol which was added on equal volume basis of extract to precipitate the pectin, and kept for two hours the ethanol caused coagulation of pectin which was recovered by filtration. The precipitate is separated, washed with more alcohol to remove impurities. The coagulated pectin was put in petridish and kept in an oven for 50 °C for 24 hours. The pectin obtained was then grinded for further analysis and for characterization purpose.

4. RESULTS AND DISCUSSION

4.1 Yield and extractability of pectin

The extraction parameters were very important to produce both a good quality and a reasonable amount of pectin. As a result to achieve these objectives different parameters with different levels were used during the extraction process. The parameters were temperature, and pH. By using literature reviews as spring point and taking into consideration the levels of temperature were 40 °C, 60 °C, and 80 °C. The second important factor and that was necessarily investigated was pH and its levels were pH 1.0, pH 2.5, and pH 4.0. Since all interaction of the level of factors should be considered in factorial design, there were twenty seven runs that could be performed during the laboratory experiment that could be found by multiplying the levels of the factors. The laboratory experimental outputs in different variable with different levels were recorded in table 4.1 and 4.2. The yield was obtained by using equation (3.1).

The percentage yield of pectin extracted by using nitric acid from lime and lemon peel powder ranged from 4.6% to 19.6% and 5.2% to 21% respectively. The percentage yield was maximum (19.6%) for lime peel powder using nitric acid at treatment combination of pH 1.0 at 60 °C for 120 min while minimum yield obtained was 4.6% at treatment combination of pH 4 at 40 °C for 120 minute. Srivastava and Malviya (2011) suggested that through conventional water based extraction method at lower pH with temperature not more than 70 °C one can find maximum amount of pectin yield. The percentage yield of pectin extracted by using nitric acid from lemon peel powder was maximum 21% at treatment combination of pH 1.0 at 60 °C for 120 min. It is recognized that an increase in acid strength (that is, decreasing pH) plays an important role in increasing the content of galacturonic acid (Yapo, 2007). Khan *et al.* (2015) found heating at 70 °C for 30 min while maintaining the pH level at 2.0-2.5 was optimum to extract highest percentage of pectin. At this temperature (70 °C), no precipitate of pectin was obtained beyond pH 4.0 even though the heating time being increased to 3 h. Kanmani *et al.* (2014) the extraction from *C.limon* resulted in a maximum yield of 36.71% at pH 3.2 and temperature of 60 °C.

In general, pectin extraction yield from different sources may vary depending on processing parameters and sample features. Acids are generally the strongest extracting agents with regards to the yield of extracted pectin (Yapo, 2009). Pectin yield greater than 10% is commercially

feasible (Mohd, *et al*., 2012). The highest yield obtained in this study was more than 10%, which makes it feasible for commercial use.

4.2 Interpretation of the results using experimental design software:

The experimental design selected for this study is general factorial and the response variable is % yield. General factorial with three-level-two-factor was chosen. The categorical factors are temperature and pH at levels 40 °C, 60 °C, 80 °C and 1.0, 2.5, and 4.0 respectively. Treatment of dried lemon peel at 40, 60, and 80 °C and pH of nitric acid (1.0, 2.5, and 4.0) were combined to evaluate the significance of the main effects (temperature and pH) or interactions of temperature and pH on percentage yield of extracted pectin.

A total of 27 experiments were carried out for each variety meaning total of 54 experiments. Having two factors instead of three was cited as desirable because it reduced the preparation time and lessened the potential for mistakes in preparing the experiments. The laboratory results of the experiment have been collected depending on the run order recommended by the Design Expert software 7.0.0 software and the result of the three replicate was taken and tabulated in the Table (4.1 and 4.2).

Analysis of variance was employed to determine the significance of the models. The test for the significance of the regression models, the test for significance on individual model coefficients, F-test and the lack-of-fit test were performed using the same statistical software package to obtain the best fit. The larger the value of F and the smaller the value of p, the more significant is the corresponding coefficient term. The coefficient of determination (R^2) is defined as ratio of sum of squares due to regression to the total sum of squares and is interpreted as the proportion of the variability in the data explained by the ANOVA (Mason, 2003) The words lack of fit refers to the fact that the simple linear regression model may not adequately fit the data. Prediction error sum of squares (PRESS) is a measure of how well the model for the experiment is likely to predict the responses in a new experiment. Smaller values of PRESS are desirable. Verification of optimized parameters and predicted values were done in triplicate to confirm the validity of the models.

The results were analyzed by applying the coefficient of determination (R^2), analysis of variance (ANOVA) and response plots. The response surfaces help to understand the effect of process

variables and parameters up on the responses and direction the response in which it increases or decreases. The actual experimental values were quantitatively compared with the predicted values to compute the prediction error (%). Linear correlation between the actual and predicted values of each response was evaluated from the error (%) to obtain the R^2 values for optimum model validation.

This experimental design software was made to screen out insignificant factors and to identify significant factors and get some idea about the existence of interaction effects. To understand main effects and get complete information about 2-factor interaction and also characterize how the significant factors affects the response (for optimization purposes).

The number of runs, number of factors, name and units of factors and their levels, the response name and units were entered and the following design summary table was generated.
The statistical software program is used to generate the model equation, interaction effects of the independent variables and surface plots using the fitted equation obtained from the regression analysis holding one of the independent variable constant.

The general factorial conditions and their respective average responses (yield) for the two varieties are given in Table (4.1) and Table (4.2) respectively. The actual percentage yield of pectin was calculated using equation 3.1 and it is tabulated in Appendix A13&14. The details and results are discussed in sections below and in Appendix.
Table 4.1 Shows average laboratory result of Lisbon variety

Run	Temperature °C	pH	Average % Yield
1	40	1	8.106
2	60	1	20.9
3	80	1	17.016
4	40	2.5	7.36
5	60	2.5	17.5
6	80	2.5	13.26
7	40	4	5.24
8	60	4	12.12
9	80	4	8.96

Table 4.2 Shows average laboratory result of Mexican variety yield

Run	Temperature °C	pH	Average % Yield
1	40	1	9.36
2	60	1	19.43
3	80	1	16.3
4	40	2.5	7.26
5	60	2.5	16.16
6	80	2.5	12.73
7	40	4	4.76
8	60	4	12.23
9	80	4	8.03

4.2.1 Development of regression model equation

The mathematical relationships between the response (pectin yield) and the independent variables temperature (A), and pH (B) in terms of coded and actual factors can be determined by Design Expert software. The model equation that correlates the response (Y) to the extraction process variables in terms of coded factors after excluding the insignificant terms was given in equation (4.1 & 4.2).

Lisbon peel pectin:

Final equation in terms of coded factors:

$$Yield = 17.27 + 3.09*A-3.28*B-1.30 * A * B-6.84*A^2-0.65*B^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.1)$$

Where, A = Extraction temperature

B = pH

Similarly on the basis of the coefficients in equations (4.3) it was evident that the percentage of pectin yield increases with the temperature (A) and decrease with pH (B). As expressed in equation (4.1) the temperature showed significantly linear effects on the pectin yield, which was clearly indicated by the largest positive linear regression coefficient 3.09 and while pH possessed negative effect with the linear coefficient (-3.28).

4.2.1.1 Model adequacy check

The model was tested for adequacy by analysis of variance. The regression model was found to be highly significant with the correlation coefficients of determination of R-Squared, adjusted R Squared and predicted R-Squared having a value of 0.9828, 0.9787 and 0.9720 respectively. The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.9828. It implies that 98.28% of the total variation in the yield of pectin is attributed to the experimental variables studied. The R^2 value of 0.9828 validates the accuracy of the model. This value provides a measure of how much variability in the observed response can be explained by the experimental factors and interaction. The closer that the R^2 value is to 1.0, the stronger the model is and the better it predicts the response. The graph of the predicted values obtained using the developed correlation versus actual values is shown in Figure (4.2).

The results in Figure (4.2) demonstrated that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the two process variables to % Yield.

The adequacy of the model was further checked with analysis of variance (ANOVA) as shown in Table (4.3). Based on a 95% confidence level, F–value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is likely that any of the factors have a significant effect on the response with the P – value less than 0.05. It is calculated by model mean square divided by residual mean square. Here the model F – value of 239.57 implies the model is significant. There is only a 0.01% chance that a "Model F – Value" this large could occur due to personal error or disturbance.

Mexican peel pectin:

Final equation in terms of coded factors:

$$\text{Yield} = 16.19 + 2.61 * A - 3.35 * B - 0.91 * A * B - 6.20 * A^2 - 0.37 * B^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.2)$$

Where, A = Extraction temperature

B = pH

Similarly on the basis of the coefficients in equations (4.2) it was evident that the percentage of pectin yield increases with the temperature (A) and decrease with pH (B). As expressed in Eq (4.2) the temperature showed significantly linear effects on the pectin yield, which was clearly indicated by the largest positive linear regression coefficient 2.61 and while pH possessed negative effect with the linear coefficient (-3.35). Clearly the coefficients in the above equation show that pectin yield increases with increase in temperature (A) and decreases with pH (B).

4.2.1.2 Model adequacy check

The model was tested for adequacy by analysis of variance. The regression model was found to be highly significant with the correlation coefficients of determination of R-Squared, adjusted R Squared and predicted R-Squared having a value of 0.9983, 0.9978 and 0.9972 respectively. The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.9983. It implies that 99.83% of the total variation in the yield of pectin is attributed to the experimental variables studied. The R^2 value of 0.9983 validates the accuracy of the model. This value provides a measure of how much variability in the observed response can be explained by the experimental factors and interaction. The closer that the R^2 value is to 1.0, the stronger the model is and the better it predicts the response. The graph of the predicted values obtained using the developed correlation versus actual values is shown in Figure (4.1).

The results in Figure (4.1) demonstrated that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the two process variables to % Yield.

The adequacy of the model was further checked with analysis of variance (ANOVA) as shown in Table (4.4). Based on a 95% confidence level, F – value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is likely that any of the factors have a significant effect on the response with the P – value less than 0.05. It is calculated by model mean square divided by residual mean square. Here the model F – value of 212.41 implies the model is significant. There is only a 0.01% chance that a "Model F – Value" this large could occur due to personal error or disturbance.

The “Adeq Precision" measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable. Here ratio of Mexican and Lisbon peel pectin 142.503 and 43.135 respectively indicates an adequate signal. Therefore, this model can be used to navigate the design space.

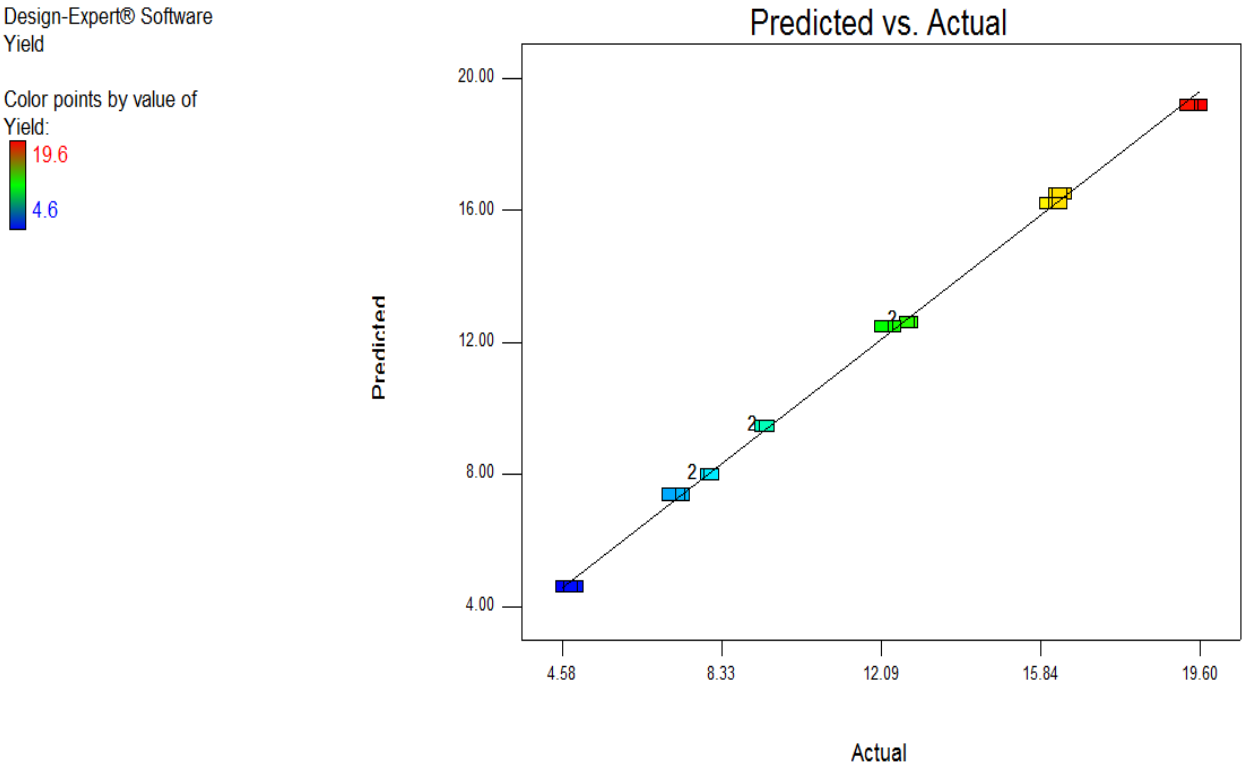


Figure 4-1 Predicted versus actual % Yield of Mexican variety

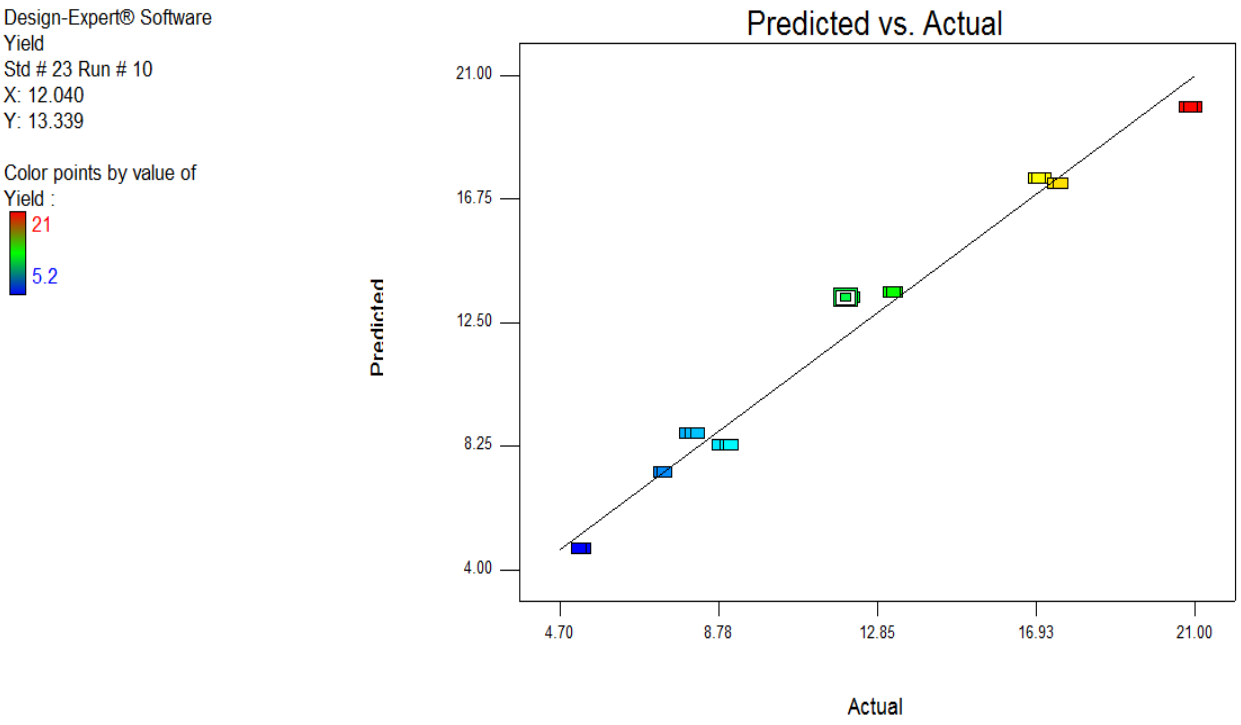


Figure 4-2 Predicted versus actual % yield of Lisbon variety

The results in Figure 4.1&4.2 demonstrated that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit and splits evenly by the 45 degree line. This result indicates that it was successful in capturing the correlation between the two process variables to the yield of pectin. The outcomes in Figure 4.1 and 4.2 demonstrated that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the two extraction process variables to the yield of pectin.

4.3 Statistical analysis on factors affecting pectin yield

The statistical analyses of the ANOVA are given in Tables 4.3 and 4.4, respectively. The actual yield of pectin at different process parameters was calculated and is given in appendix A. The model was tested for adequacy by analysis of variance. The statistical analysis of the ANOVA for the response value pectin yield was analyzed using DoE and the result is given in Tables 4.3 and 4.4.

Table 4.3 Analysis of variance (ANOVA) for regression model equation and coefficients for Lisbon peel pectin

Source	Sum of squares	Df	Mean Square	F Value	p-value prob>F	
Model	680.89	8	85.11	8622.85	<0.0001	Suggested
A-temp	452.71	2	226.36	22932.95	<0.0001	
B - pH	196.44	2	98.22	9951.00	<0.0001	
AB	31.73	4	7.93	803.73	<0.0001	
Pure total	0.18	18	0			
Cor total	681.06	26				

The model F- value of 8622.85 implies the model is significant. There is only a 0.01% chance that a “Model F-value” this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, and AB are significant model terms. This shows that the temperature, pH, interaction temperature and pH, square of temperature and square of pH affect the % yield significantly.
Each one and combined factor effects were shown more significance. The significant effects of the factors were determined using the analysis of variance and the predicted versus actual graph. From the ANOVA table 4.3, all the factors have values of “prob > F” less than 0.05. From design expert, this indicates that all the factors were significant during the extraction of pectin.

Table 4.4 Analysis of Variance (ANOVA) for the regression model equation and coefficients Mexican peel pectin

Source	Sum of squares	Df	Mean Square	F Value	p-value prob>F	
Model	566.60	8	70.82	4682.32	<0.0001	Suggested
A-temp	353.55	2	176.77	11686.56	<0.0001	
B - pH	202.28	2	101.14	6686.56	<0.0001	
AB	10.70	4	2.69	177.92	<0.0001	

Pure total	0.27	18	0.015			
Cor total	566.87	26				

The model F- value of 4682.32 implies the model is significant. There is only a 0.01% chance that a “Model F-value” this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, and AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

This shows that the temperature, pH, interaction temperature and pH, square of temperature and square of pH affect the % yield significantly. Each one and combined factor effects were shown more significance. The significant effects of the factors were determined using the analysis of variance and the predicted versus actual graph. From the ANOVA table 4.4, all the factors have values of “prob > F” less than 0.05. From design expert, this indicates that all the factors were significant during the extraction of pectin.

4.3.1 Normal probability plot of the residuals

The normal probability plot of the residuals showed that, it follows almost a straight line which implies residuals are approximately normally distributed that satisfy the most important assumption in any model.

Figure 4.3 and 4.4 shows how precisely the pectin yield is modeled, because all the points line up well and the deviation of points for pectin yield from normality is insignificant. In addition, the normal probability plot indicates the residuals following a normal distribution, in the case of this experiment the points in the plots shows fit to a straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

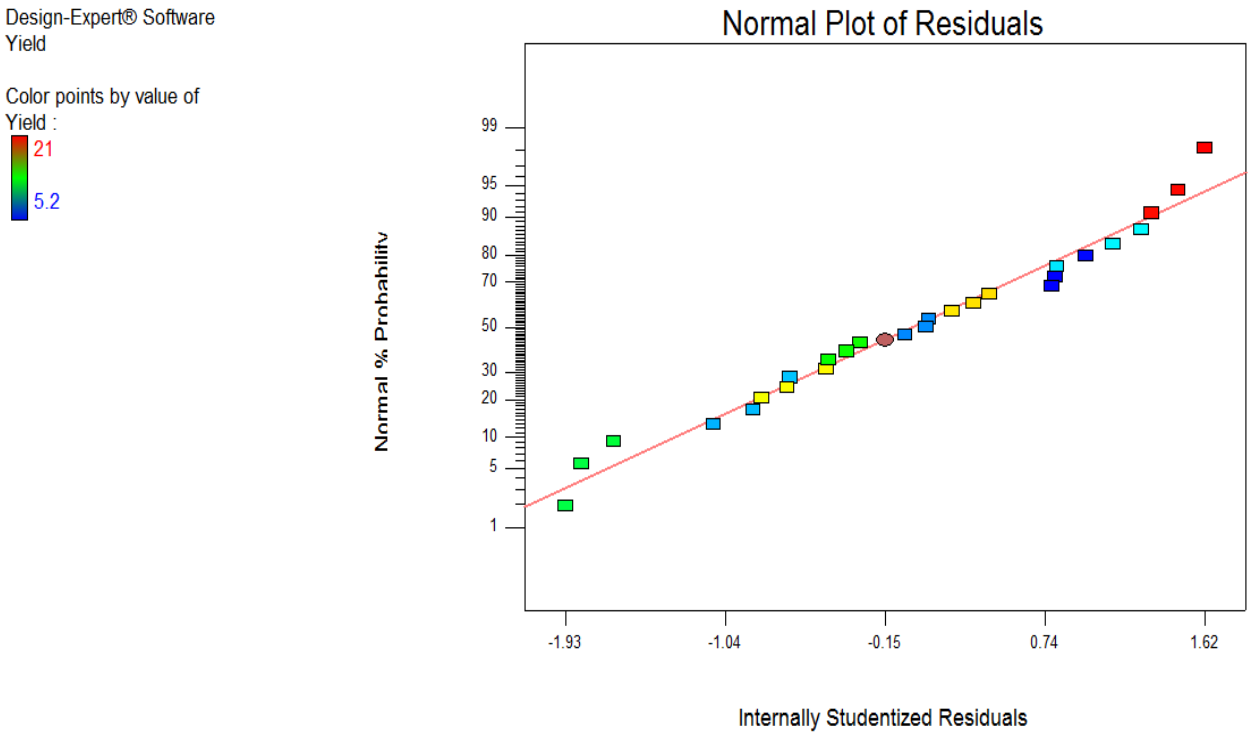


Figure 4-3 Normal plots of residuals yield of Lisbon peel pectin

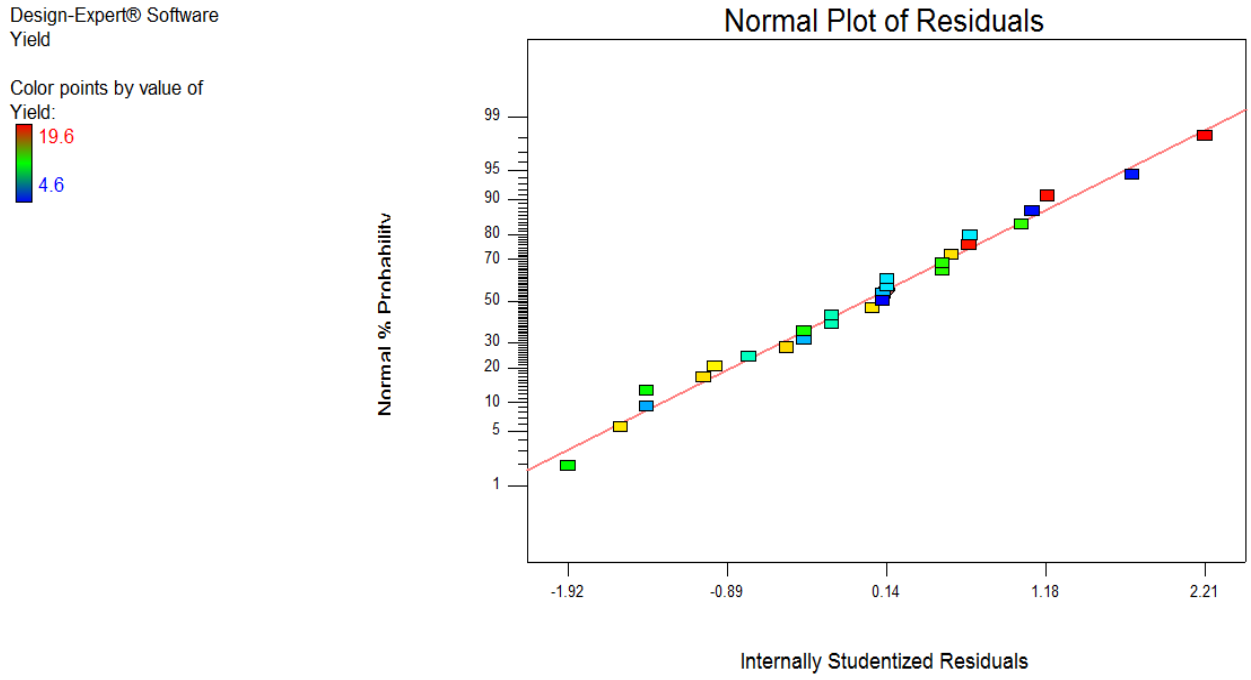


Figure 4-4 Normal plots of residuals yield of Mexican peel pectin

4.3.2 Plot of residuals verses predicted values

Figure below showed that the plot of residuals verses predicted value did not follow any pattern, it is random it implies that the model is adequate.

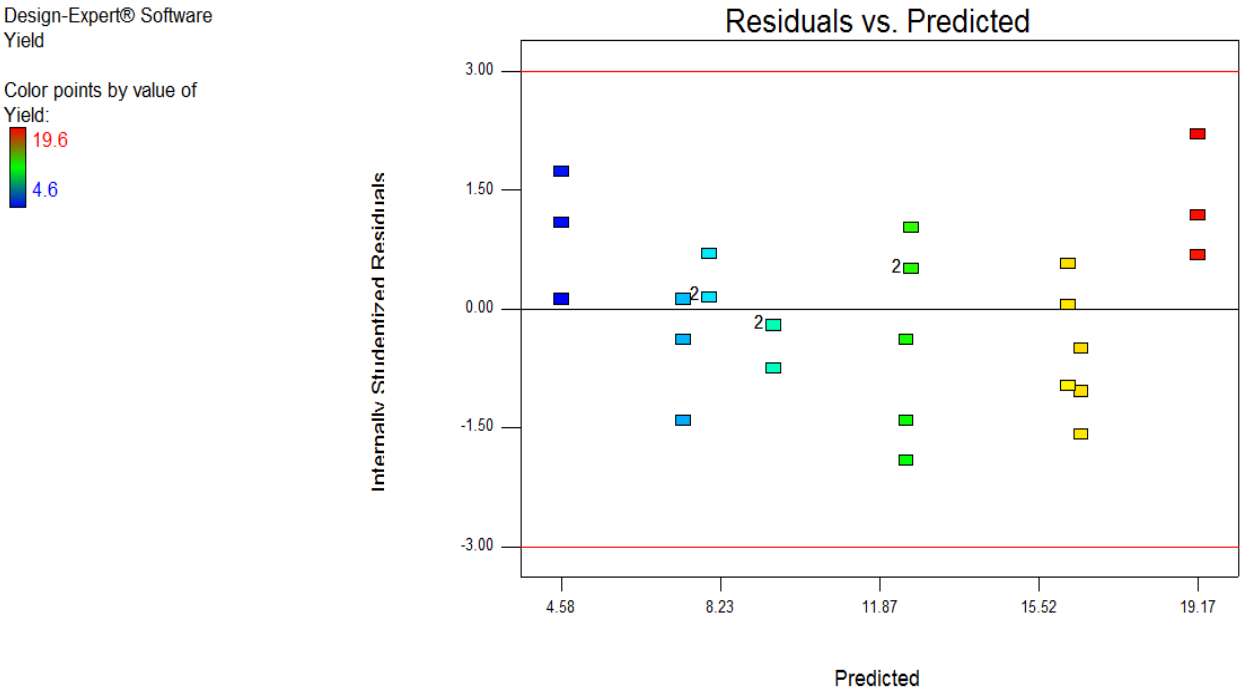


Figure 4-5 Plots of residuals vs predicted values of Mexican lime

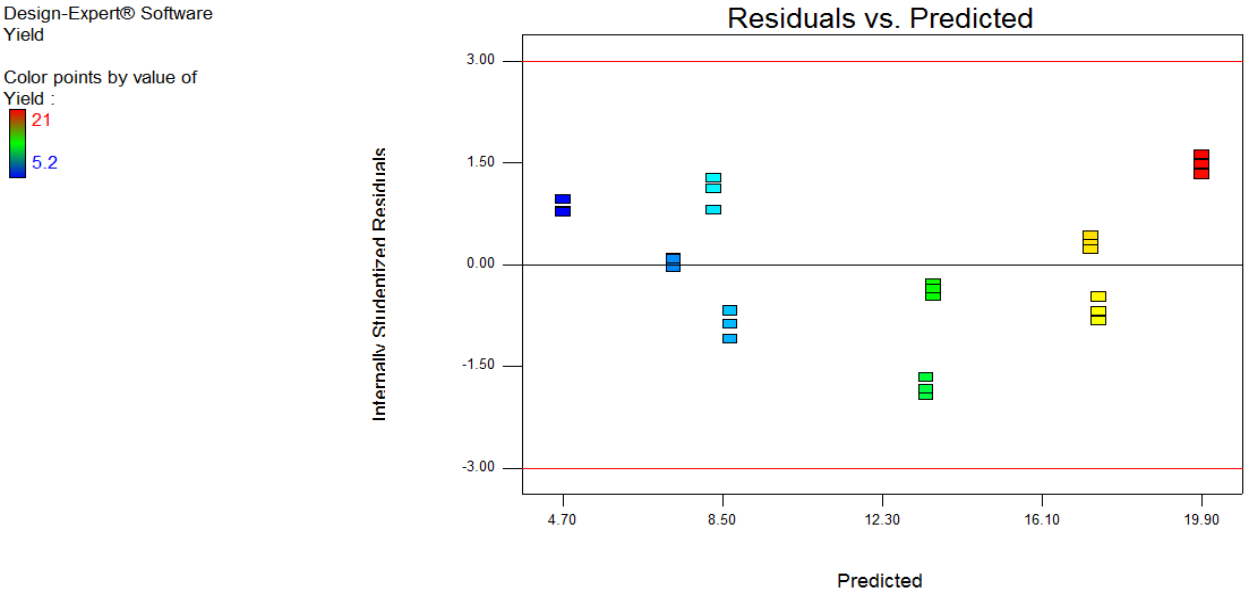


Figure 4-6 Plots of residuals vs predicted values of Lisbon

4.4 Effect of individual process variables

Based on ANOVA we have seen that temperature and pH significantly affect the percentage yield of pectin.

4.4.1 Effect of pH on pectin yield

With regards to the extraction parameters, pH is considered as one of the most crucial parameters affecting both the amount and properties of pectin being extracted. It has previously been reported that several acids can be utilized for the extraction of pectin. However, mineral acids such as sulfuric, hydrochloric and nitric acids tend to be widely used (Visser & Voragen, 1996). Therefore, in this study, the effects of pH on pectin yield were initially investigated. As shown in Table 4.1 and 4.2, it is evident that pectin extraction at pH 1 gave significantly higher yield than that of pH 2.5, and 4. This is likely due to the enhanced ability of acid in solubilizing the proto pectin from the albedo with an increase of acid strength. This observation is in good agreement with the previous works of Khan *et al*., (2015) and Sharma *et al*., (2006), which reported significant influences of acid concentration on yields. Therefore, nitric acid at pH 1 would be preferential in view of pectin extraction from both varieties. Acids affect not only the yield but also the molecular characteristics of pectins such as DE, gelling properties, ratio of galacturonic acid to rhamnose, average molecular weight and gel strength (Liu *et al*., 2009).

Acids are generally the strongest extracting agents with regards to the yield of extracted pectin (Yapo, 2009). Using nitric acid at pH 1 yield of 19.6 was obtained from lime and 21% yield from lemon at the same processing conditions.

4.4.1.1 Effect of pH on Mexican variety

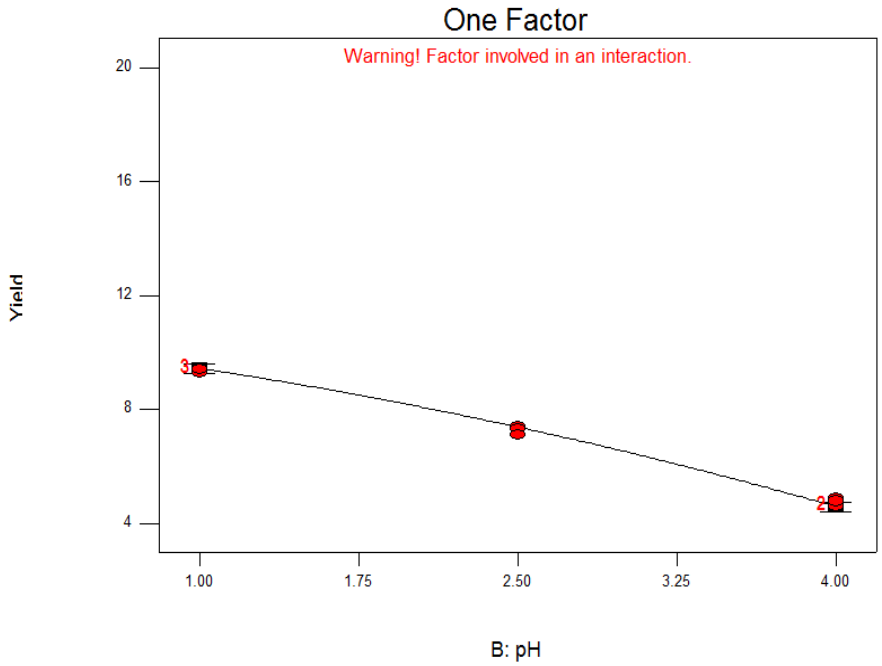
Design-Expert® Software

Yield

Design Points

X1 = B: pH

Actual Factor
A: Temperature = 40.00



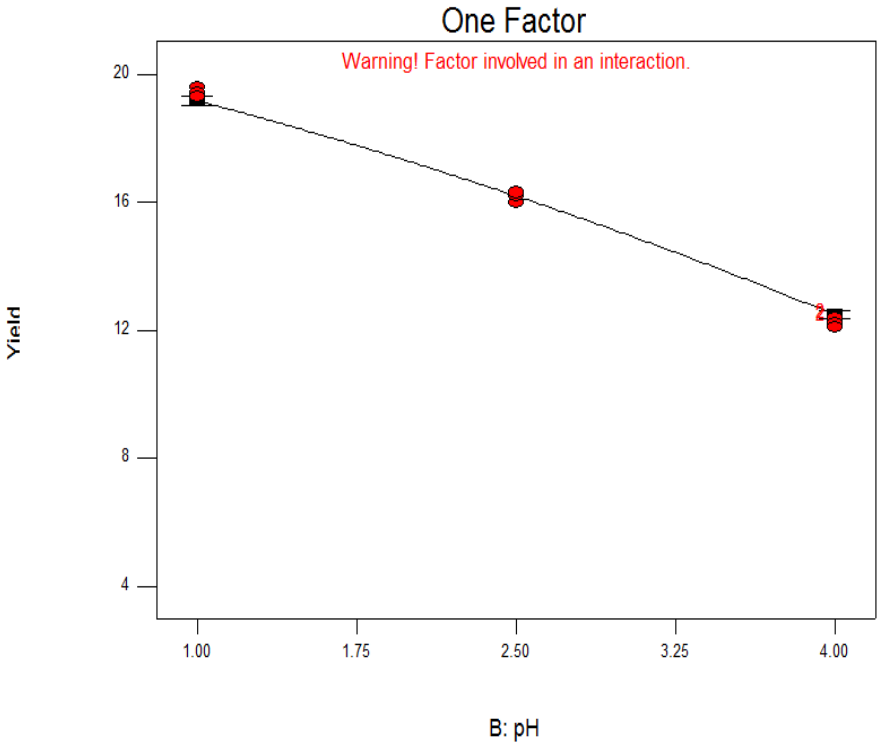
Design-Expert® Software

Yield

Design Points

X1 = B: pH

Actual Factor
A: Temperature = 60.00



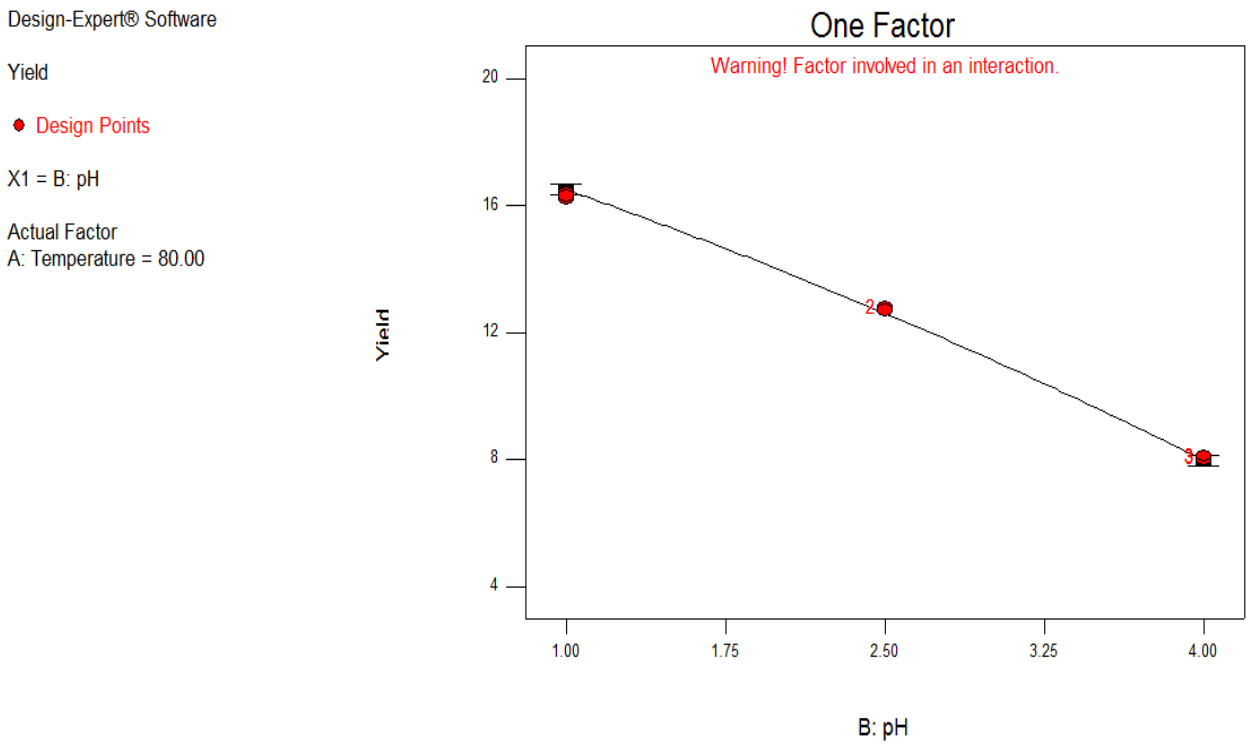


Figure 4-7 Pectin yield versus pH Mexican variety at different temperatures

The above three figures from design expert software clearly shows the change in the yield of pectin at different pH while the temperature kept constant. As the pH strength decreases from 1-4 pectin yield also decreases for all temperatures (Fig 4.10). Minimum yield was obtained for three temperatures at pH 4and maximum yield was obtained at pH 1 for all temperatures. At pH 1 we have maximum pectin yield 9.4, 19.6, and 16.4% for temperatures 40, 60, and 80°C respectively. Altaf *et al.* (2015) using HCl from papaya peel observed that with increase in pH pectin yield decreased.

4.4.1.2 Effect of pH on Lisbon variety

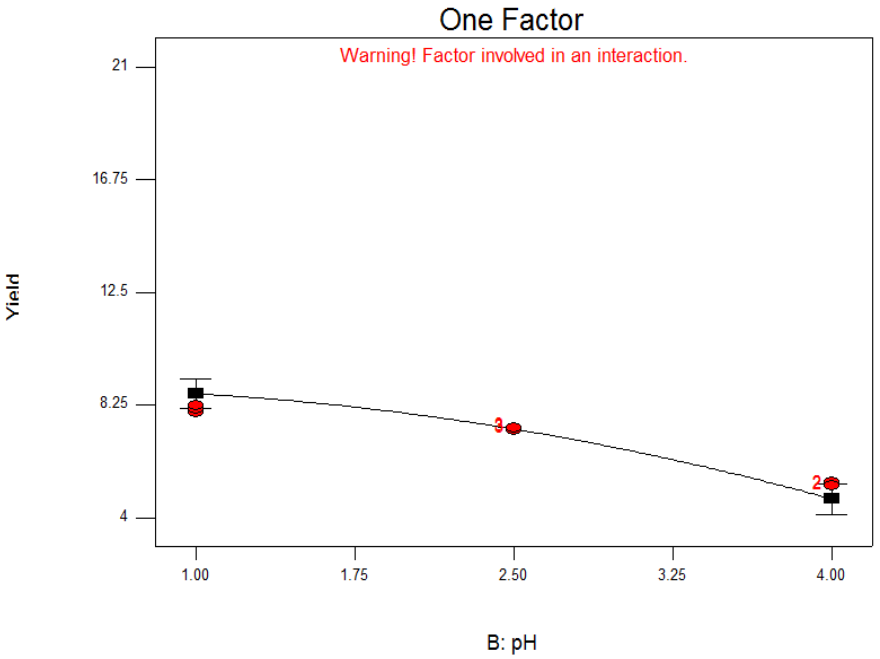
Design-Expert® Software

Yield

Design Points

X1 = B: pH

Actual Factor
A: Temperature = 40.00



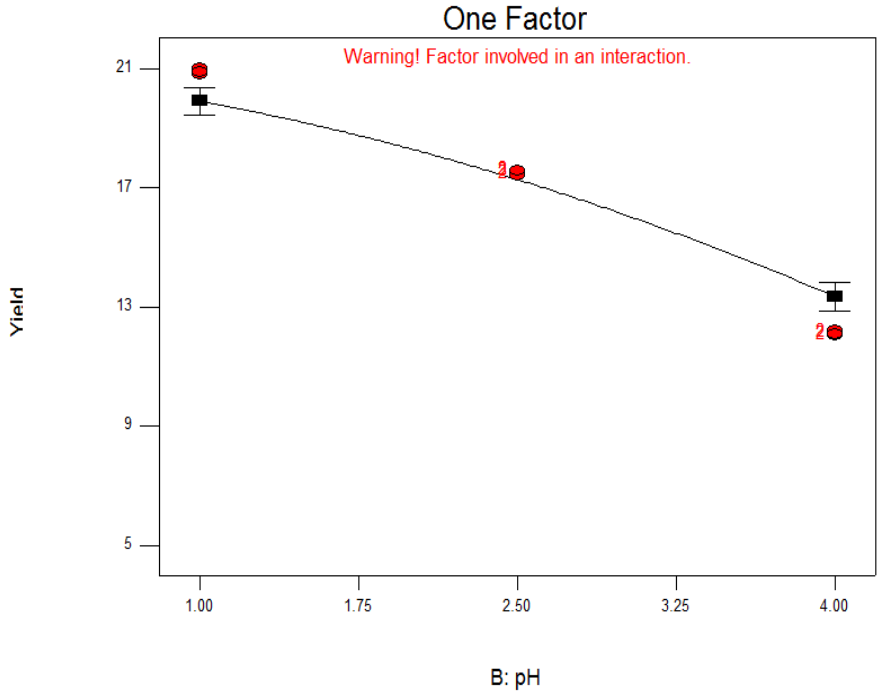
Design-Expert® Software

Yield

Design Points

X1 = B: pH

Actual Factor
A: Temperature = 60.00



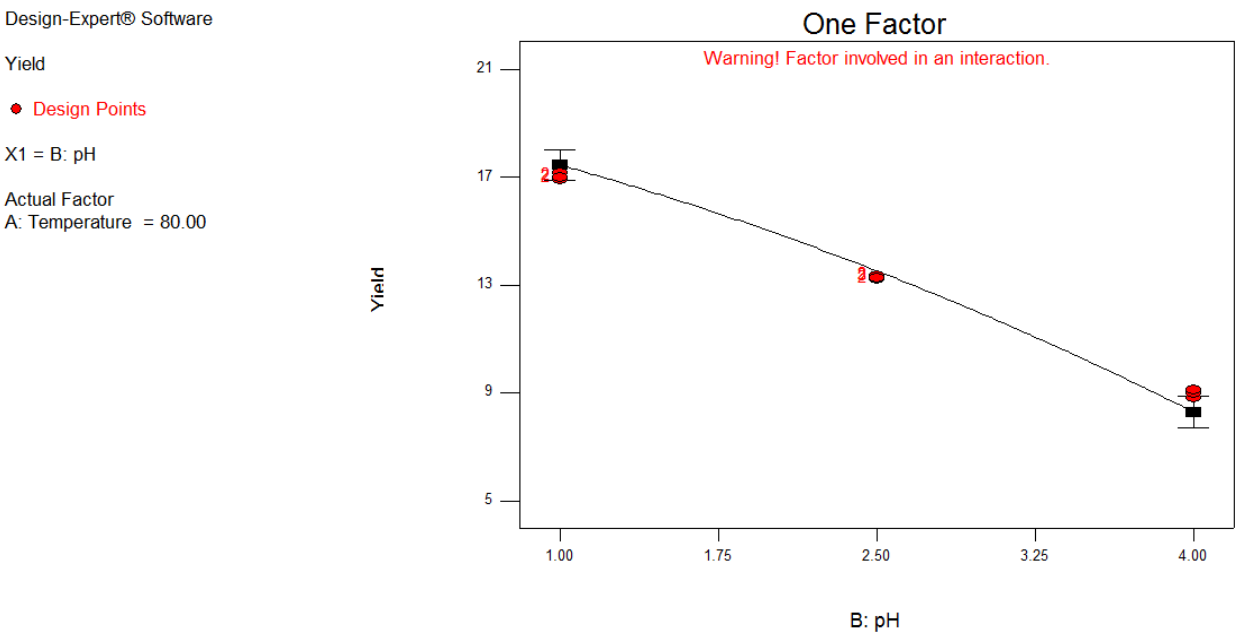


Figure 4-8 Pectin yield versus pH Lisbon variety at different temperatures

The above three figures from design expert software clearly shows the change in the yield of pectin at different pH while the temperature kept constant. At temperature 40 °C and pH 1pectin has highest yield of 7.97%. As the pH increases from 1-4 the yield continuously decreases. Both at temperature 60 °C and 80 °C pH shows similar behavior that the yield continuously decreases from pH 1-4. The above figure depicts that at pH 1 and temperature 60 °C we can find maximum yield of 21%. It also shows us if we increase the pH the yield will decreases.

4.4.2 Effect of temperature on pectin yield

Figure 4.4 shows as the extraction temperature increases pectin yield also increased. But some literatures suggest that increased temperature beyond 80 °C may denature pectin and it is better to extract it below 80 °C for maximum yield. Srivastava and Malviya (2011) suggest that extracting pectin using acidified water (pH up to 2) at temperature not more than 70 °C could give maximum yield. For both varieties maximum yield was obtained at temperature 60 °C. Similar result has been observed by kanmani *et al.* (2014) the optimum extraction temperature for *C. limon* was 60 °C.

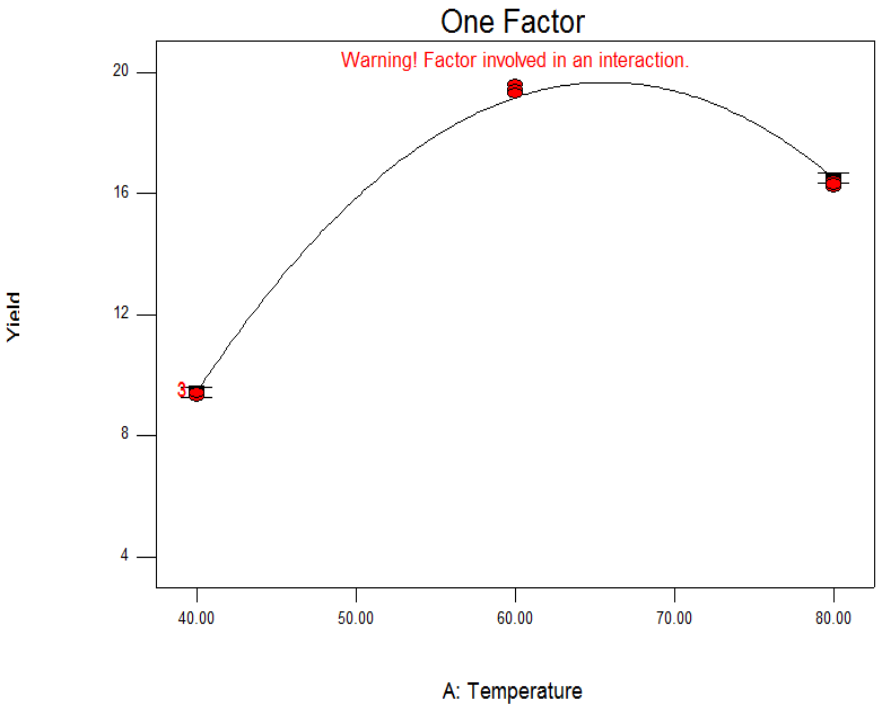
Design-Expert® Software

Yield

Design Points

X1 = A: Temperature

Actual Factor
B: pH = 1.00



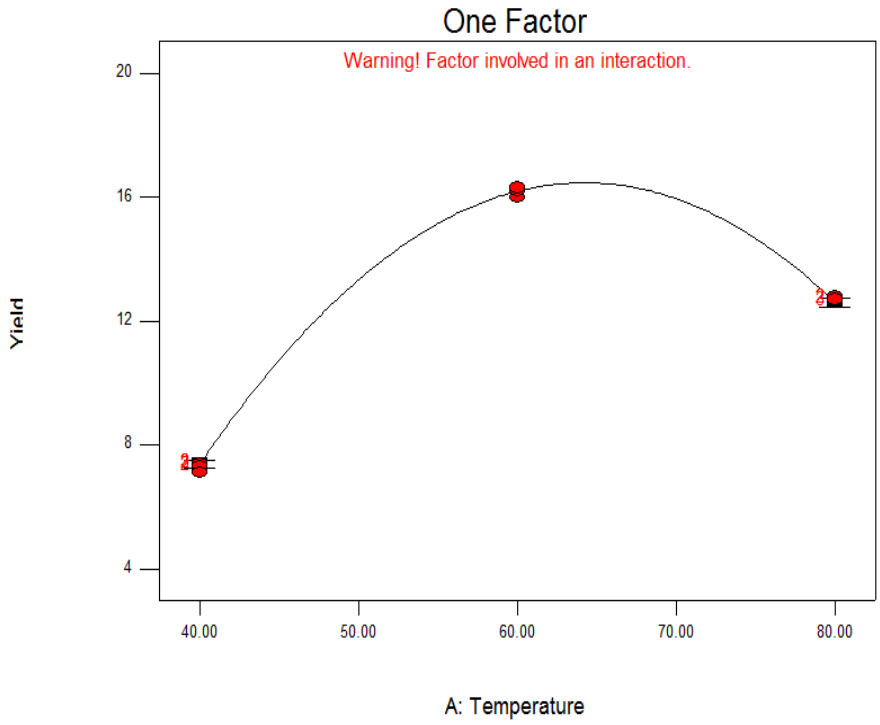
Design-Expert® Software

Yield

Design Points

X1 = A: Temperature

Actual Factor
B: pH = 2.50



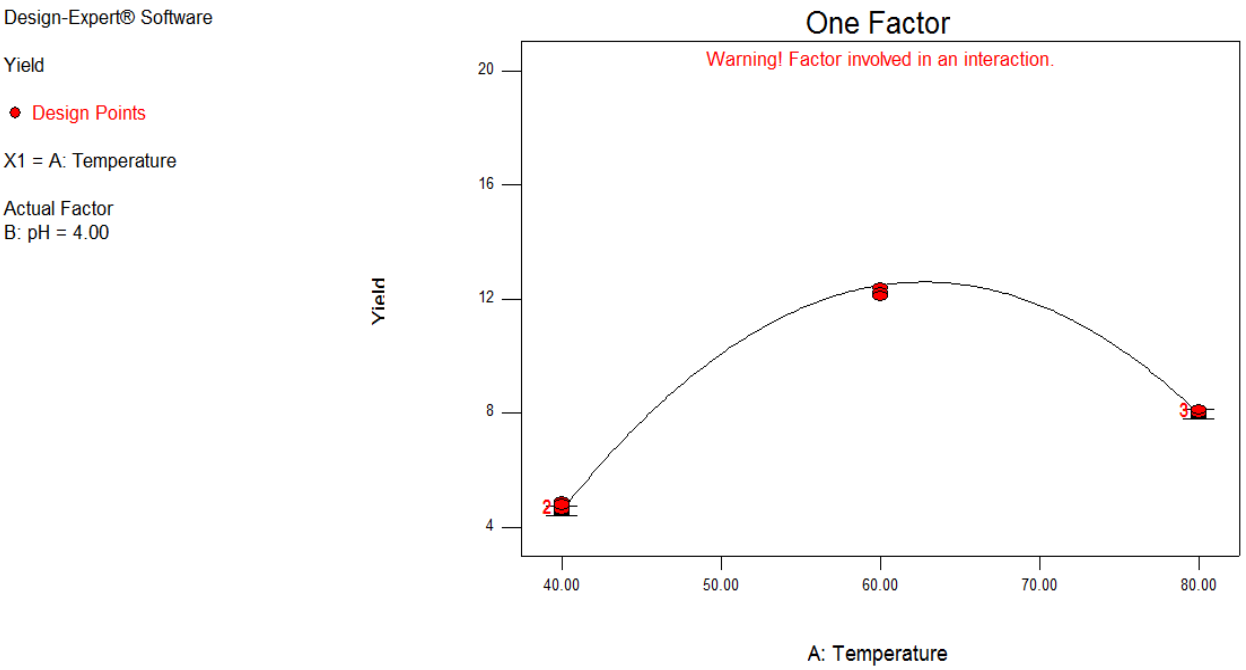
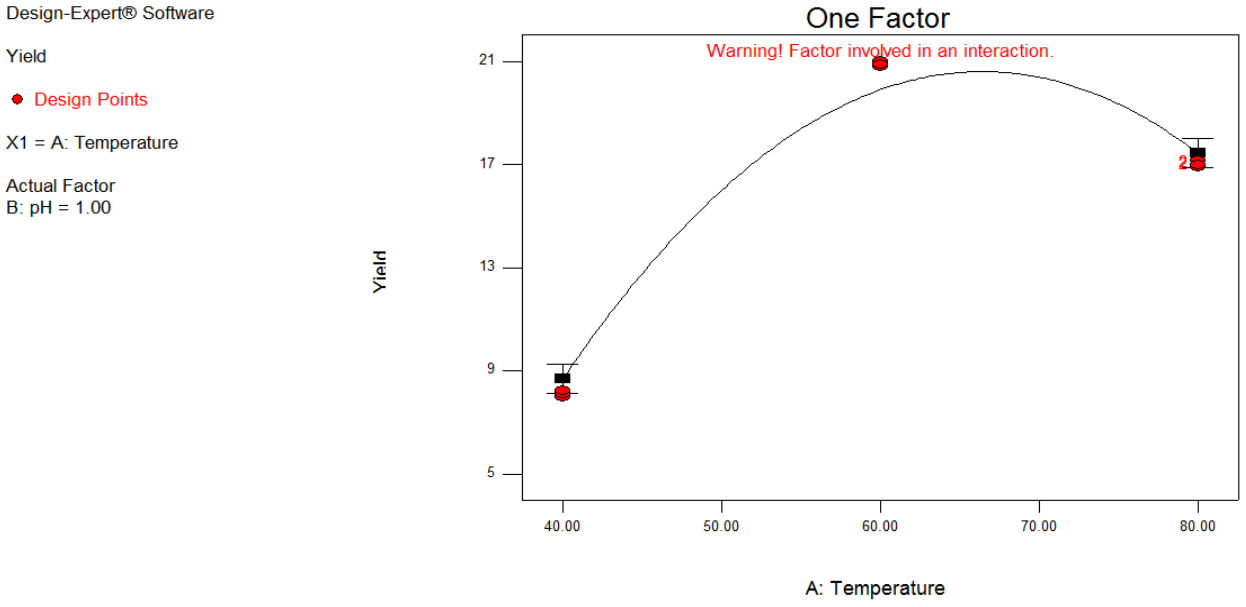


Figure 4-9 Pectin yield versus temperature at various pH for Mexican variety

The above three figures from design expert software clearly shows the change in the yield of pectin at different temperature while the pH is kept constant. The figures show that minimum yield is obtained at temperature of 40 °C for all pH. The figures also predict maximum yield could be found from the temperature 60 °C and as temperature increased beyond 60 C, pectin yield also increased up to some point and decreased until 80 °C.



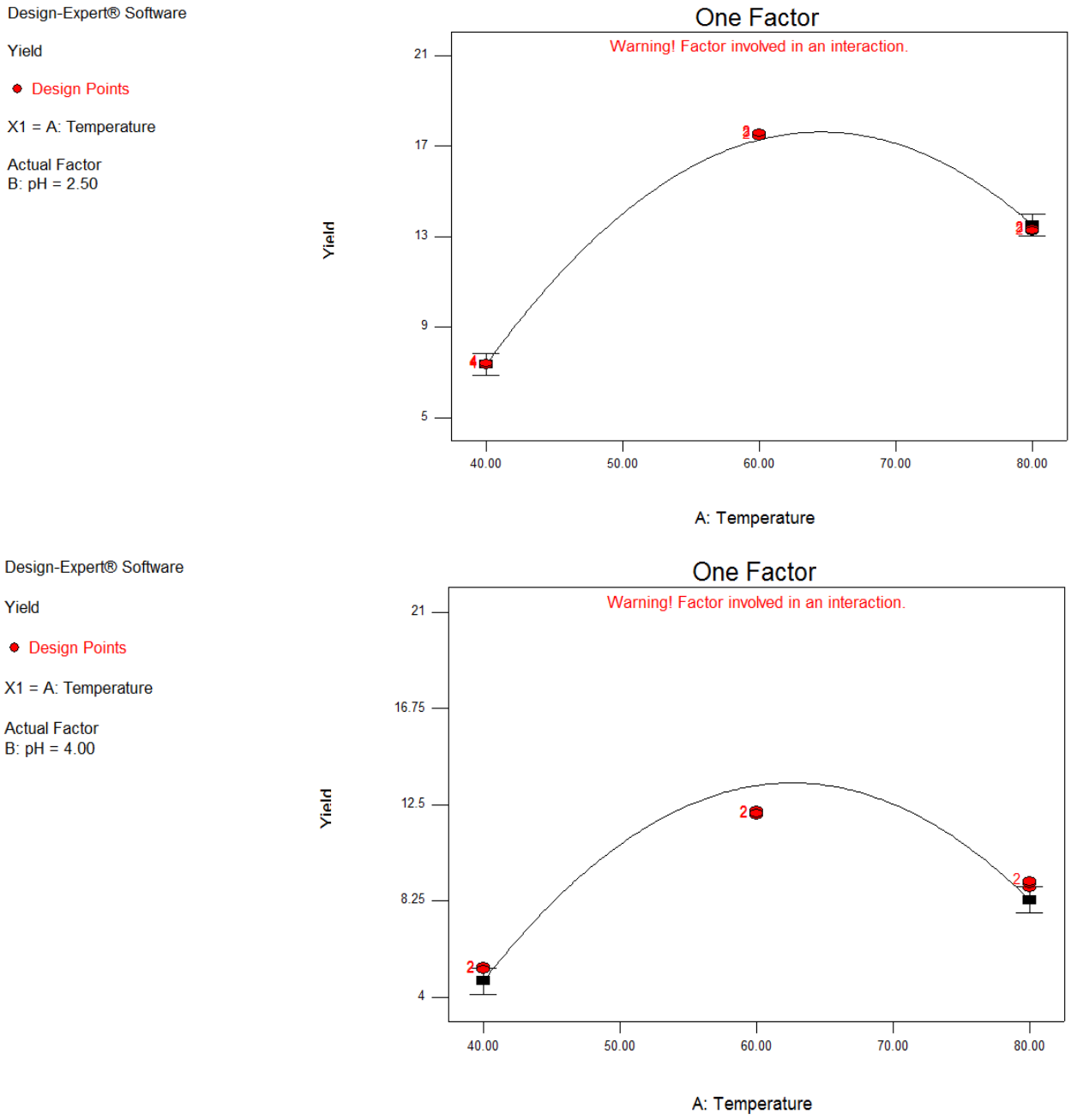


Figure 4-10 Pectin yield versus temperature at various pH for Lisbon variety

In the above figure at temperature 60 °C and at pH=1 we have maximum yield of 21%. Clearly from the figure one can observe that as the temperature increase beyond 65 °C pectin yield decreases. The above three figures from design expert software clearly shows the change in the yield of pectin at different temperature while the pH is kept constant. The figures show that minimum yield is obtained at temperature of 40 °C for all pH. The figures also predict maximum yield could be found from the temperature 60 °C up to 70 °C.

4.5 Effect of interactive parameters between process variables

4.5.1 Effect of temperature and pH

The effects of temperature and pH on pectin yield are show in the form of 3D plots and surface contour. The influence of the temperature and pH at fixed time on the pectin yield. The response corresponding to the contour plots of second order predicted model indicated that, for lower temperature the yield of pectin decreased with increasing pH. At lower and higher levels of both pH and temperature decreased the yield of pectin was observed. At low pH and higher extraction temperature it was found that there was increased on percentage of pectin yield. However, the pectin degraded and the color of pectin became deep when the pH value was too low and lower extraction rate when pH value was too high.

Effect of temperature and pH on Mexican variety

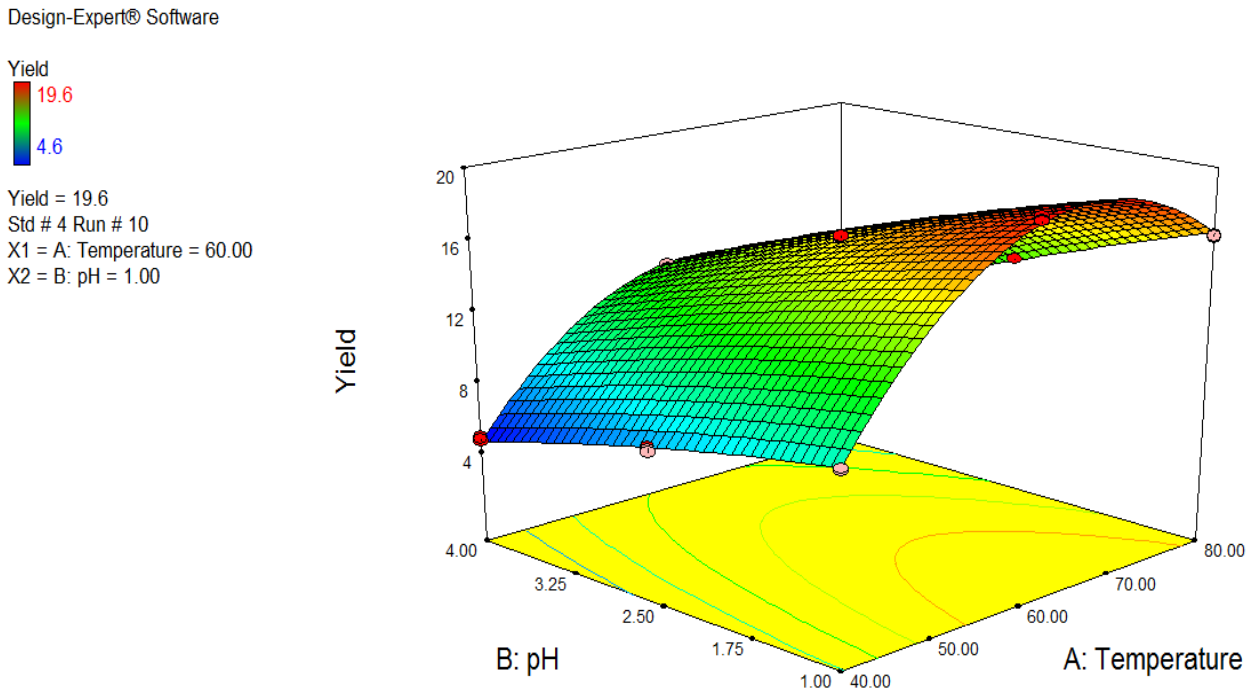


Figure 4-11 3D surface drawing showing effect of temperature and pH of Mexican Variety

The above 3D surface drawing clearly shows maximum yield is obtained at pH 1 for all temperatures as compared to other pH. Minimum yield of 4.6 % was obtained pH 4, temperature 40°C in order to get maximum pectin yield one has to operate at temperature between 60 and 80

°C at pH from 1-2.5. The plot also shows that as the pH increase (reduction in acidity of solution) % yield decreases for all temperatures.

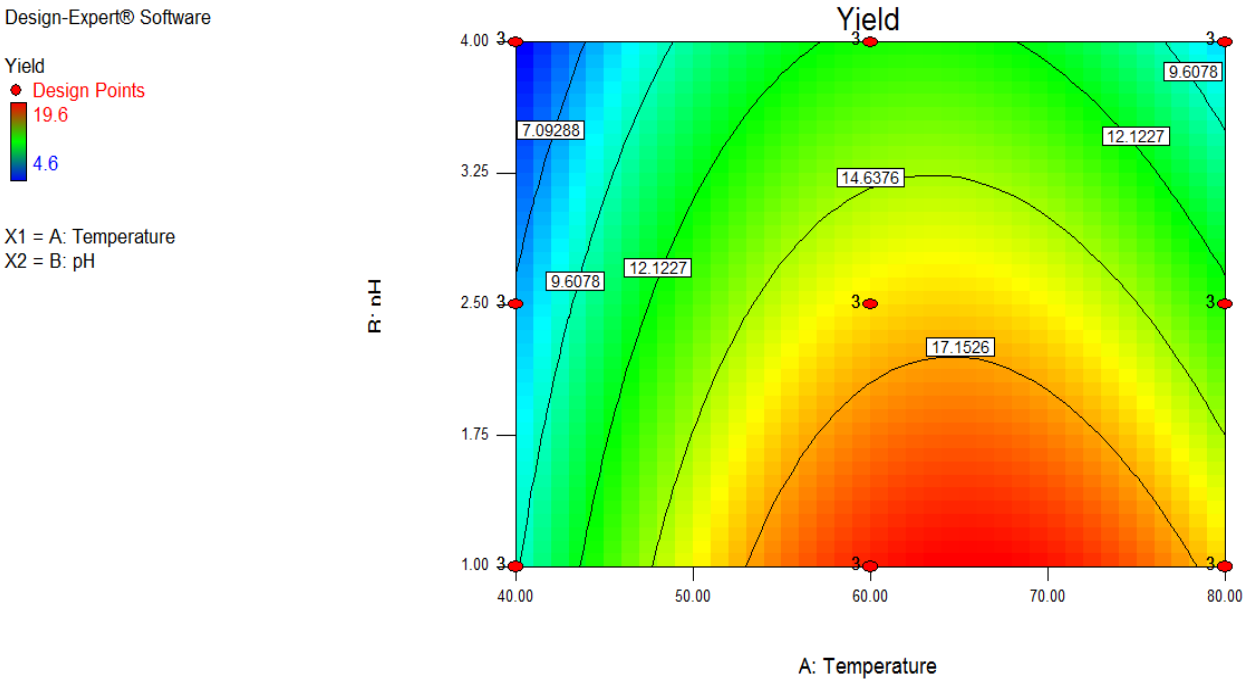


Figure 4-12 Contour showing effect of temperature and pH on Mexican variety

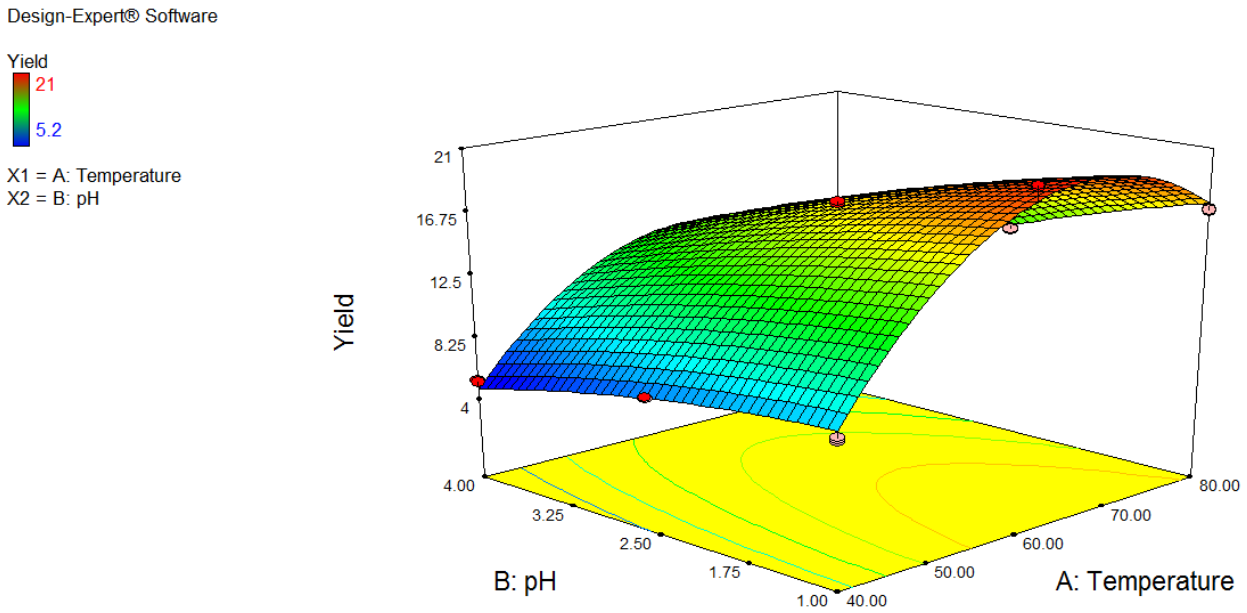


Figure 4-13 3D surface drawing showing effect of temperature and pH on Lisbon variety

The above 3D surface drawing clearly shows maximum yield is obtained at pH 1 for all temperatures as compared to other pH. Minimum yield of 5.2% was obtained pH 4, temperature 40°C. in order to get maximum pectin yield one has to operate at temperature between 60 and 80 °C at pH from 1-2.5. The plot also shows that as the pH increase (reduction in acidity of solution) % yield decreases for all temperatures.

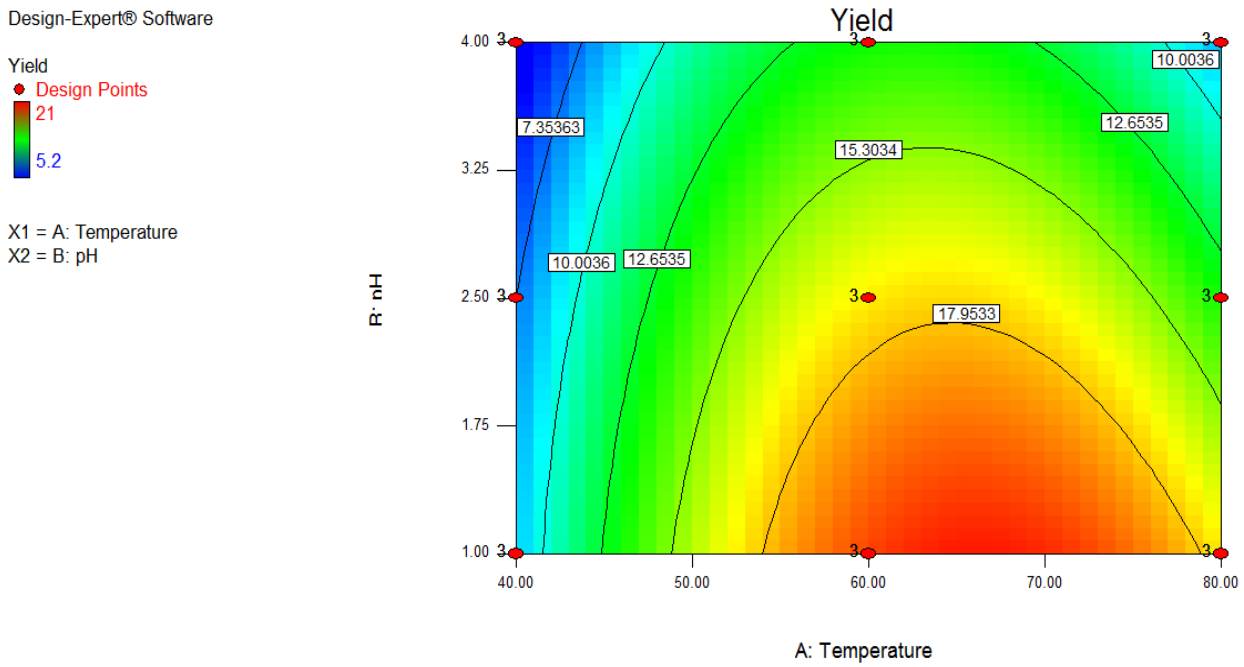


Figure 4-14 Contour showing effect of temperature and pH on Lisbon variety

4.6 Optimization of the yield of pectin

4.6.1 Optimization

Optimization may be interpreted as the way to find those values of controllable independent variable that give the most desired value of the dependent variable. Numerical optimization was carried out considering each value of the response and the goal is to maximize pectin yield.

The objective here was to obtain maximum yield in the given interval of the investigated independent variables. Using design expert software for both varieties the maximum yield of pectin was achieved at the combination of the second level of the first factor 60 °C, the first level of the second factor pH 1 and the desirability equal to 1. The minimum yield of pectin was obtained at the combination of the first level of the first factor 40 °C, the third level of the second

factor pH 4 and the desirability was equal to 1. The other analysis such as main and interaction effect analysis also enforce this conclusion

Based on the results discussed in the two factor experiments, pectin extraction with nitric acid at pH 1 resulted in the highest yield, therefore, it was consequently performed in order to study the effects of two variables, namely extraction temperature (A), and pH (B) on the response (pectin yield). Twenty seven experiments were conducted for each variety according to the conditions indicated in Table 4.1 and 4.2. Results of the response values, i.e., yield, were shown in the last columns of the same tables. Furthermore, the analysis of variance (ANOVA) results of the quadratic regression model for the pectin yield are given in Table 4.3 &4.4. It demonstrates that the model is significant with its F-value of 2399.44 and 239.15 for the Mexican and Lisbon varieties. The fit of the model was also expressed by the determination coefficient, R^2 0.9983 and 0.9828, which indicated that 98.83% and 98.28% of the variability in the response were explained by the model for Mexican and Lisbon variety respectively. In the other word, only 1.17% and 1.72% of the total variations were not explained by the model. All extraction parameters studied, the extraction temperature and pH played the most dominant role in the extraction of pectin from the two varieties ($p < 0.05$) whereas the interaction between two factors, and all quadratic parameters were also significant.

4.7 Physicochemical properties of extracted pectin

Ash content: - Ash content of pectin extracted from lemon, and lime peel powder varied from (2.45-2.81%), and (3.11-3.56%) respectively, by using nitric acid. Ranganna (1977) stipulated that the pectin of high ash content contains about 10.69% ash content and the pectin of low ash content contains about 0.76% ash content. Therefore ash content from the two citrus varieties is on the appropriate range.

Table 4.5 physicochemical properties of pectin from Lisbon variety

Temperature	pH	Ash Content (%)	Moisture content (%)	Equivalent Weight	Methoxyl content (%)	Anhydrounic acid content (%)	Degree of esterification
60 °C	1	2.45	10.2	1515.15	6.944	71.04	77.24
80 °C	1	2.67	10.31	980.4	6.14	67.89	51.34
60 °C	2.5	2.81	9.9	1428.57	6.882	51.32	76.13

Table 4.6 physicochemical properties from Mexican variety

Temperature °C	pH	Ash Content (%)	Moisture Content (%)	Equivalent Weight	Methoxyl Content (%)	Anhydrouronic Acid content (%)	Degree of esterification
60 °C	1	3.29	8.89	340.1	3.968	74.272	30.33
80 °C	1	3.56	8.7	396.82	3.41	63.712	27.62
60 °C	2.5	3.11	8.67	326.79	4.154	77.4	30.45

Moisture content

The moisture content was determined by (AOAC, 2000) using equation (3.3). The moisture content of pectin extracted from Mexican lime was between (9.9-10.2%) and that of Lisbon was between (8.67-8.89%). Based on the quality standards of commercial pectin, all of pectin is produced to meet the standards not far above 12%. The moisture content of the two varieties is below the range and it's acceptable.

Equivalent-weight

The equivalent weight of pectin extracted from lime and lemon was 326.79 between 396.82 and (980.4-1428.57) respectively. Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin (Ranganna, 1995). Pectin produced at low pH has higher equivalent weight, because low pH can cause polymerization of pectin into a

longer chain, and in turn decreases the free acid content, reported by Rouse (1977). High equivalent weight would have higher gel forming effect. Lower equivalent weight could be higher partial degradation of pectin. The determination of methoxyl and AUA contents and the equivalent weight were conducted following the method described by Owens et al. (1952). The values of equivalent weights were used for calculating the anhydrouronic acid (AUA) content and the degree of esterification.

Methoxyl content

Methoxyl content is defined as the number of moles of methyl alcohol in 100 mol galacturonic acid. Methoxyl content of pectin is important to control the gel strength, the setting time, the sensitivity to metal ions and to determine the functional properties of pectin solutions and pectin gel texture (Constenla and Lozano, 2003). Methoxyl content of lime peel pectin derived varies (3.41-4.154%) and of lemon peel powder pectin varies from (6.14-6.944%). The methoxyl content of pectin usually varies from 0.2-12% depending on the source and mode of extraction. Since all the values obtained experimentally were below 7%, the pectins are of low ester characteristic, indicating that they are desirable in terms of quality (Kanmani et al., 2014).

Anhydrouronic acid content

The results showed that the Anhydrouronic Acid content of pectin extracted from Mexican varied from (63.712-77.44%) as compared to that of pectin extracted from Lisbon (51.34-67.89%). The Anhydrouronic Acid content will be higher by increasing time of extraction. A minimum value of 65% AUA for commercial pectins has been specified by FAO.

The content of AUA indicates the purity of the extracted pectin and is suggested to be not less than 65% (Food Chemicals Codex 1996). However, the AUA content obtained from Mexican and Lisbon peel powder pectin was > 65%. Result indicates that the extract is sufficiently pure due to the possible absence of proteins, starch and sugars in the precipitated pectins.

Degree of esterification (DE)

The Degree of Esterification (DE) is the ratio of the esterified galactouronic acid groups to the total galactouronic acid groups present. It is an important property which determines the gelling nature of pectin. The results showed that pectin extracted from lemon has higher DE (77.24-51.34) as compared to lime extracted pectin (27.62-30.2). Degree of esterification of pectin can be different depending on ripeness, part of fruit, botanical origin and isolation method (Bonrood *et al.* 2005).

The types of pectin determine the mechanism for gel formation. LMP can form gels with the addition of low amount of sugar or without sugar in divalent cations. There can be an extensive range of DEs dependent on species, tissue, and maturity. A higher DE causes more rapid setting. Rapid-set pectins which are pectin with a DE of above 72% also gel at lower soluble solids and higher levels than slow-set pectins which is pectin with a DE of 58-65%.

The pectin from Mexican lime can be categorized as low methoxyl pectin (LMP) because it has a % DE that is lower than 50% whereas pectin from Lisbon variety can be categorized as high methoxyl pectin because it has % DE that is greater than 50%.

The pectins obtained from Lisbon had a medium to high degree of esterification (above 50%) and hence can be considered to be of great economic interest, as per the conventional classifications (Thakur *et al*., 1997).

4.8 Structural analysis

The FTIR spectra were used to obtain information on chemical structure. Fourier transform infrared data were obtained using on Spectrum 65 FT-IR (Perkin Elmer) in the range 4000-400 cm^{-1} using KBr pellets at faculty of natural science at department of chemistry.

4.8.1 Fourier Transform Infrared (FTIR) spectra of lime and lemon pectin

The Fourier Transform Infrared (FTIR) spectra show the functional groups and provides structural information about the extracted lemon and lime fruit pectin obtained from the different varieties in the wavelengths between 400 and 4,000 cm^{-1} (Figure 4.15&4.16). The major functional groups in pectin are usually in the region between 1,000 and 2,000 cm^{-1} of the FTIR

spectra (Kalapathy & Proctor 2001). The chemical structure of the pectin obtained from two different varieties was investigated by FT-IR as shown in (Figure 4.15&4.16).

The spectral data obtained were analyzed by comparing the FTIR spectra in the following characteristic regions: O-H stretching band envelope (3100-3600 cm^{-1}); C-H stretching bands (2800-3000 cm^{-1}) (Kamnev *et al*., 1998).
One can also notice somewhat weaker intensity of C-H stretching bands at 3040cm^{-1} for Lisbon variety pectin, which may be attributed to an essentially lower degree of methyl esterification of carboxyls in lime acid-extracted pectin as compared to Mexican variety which was at 3506 cm^{-1}. O-H stretching bands were observed at 3506 and 3441 cm^{-1} for Mexican and Lisbon varieties respectively.

However, FT-IR spectra of Mexican pectins were much like that of pectin from Lisbon variety, but distinguishably different in comparison to apple pectin in absorbance. For example, two higher peaks for apple pectin are shown at wave number 1012 cm^{-1} and 1106 cm^{-1} than other pectins. In addition, its peak at 1740 cm^{-1} was higher than peak at 1630 cm^{-1} while the other pectins unanimously show lower peak at 1740 cm^{-1} than at 1630 cm^{-1}. The differences may be contributed by the biological variation in plant material used (Liang *et al*., 2012). However, FT-IR spectra of Mexican pectins were much like that of pectin from Lisbon variety.

The FTIR spectrum of Mexican and Lisbon extract pectin is presented in Figures below and the corresponding functional groups are given in Table 4.7. (Kanmani et al., 2014) observed *C. limon* pectin exhibits sharp and strong peaks at 3595.31 cm^{-1} as O-H stretch, C-H stretch in the frequency 3000-2829 cm^{-1}whereas the Lisbon extract exhibits sharp and strong peaks at 3441 as O-H stretch and C-H stretch in the frequency range 3000-2849 cm^{-1}. Strong C=O stretch occurring at 1700-1636 and 1712-1633 cm^{-1} for Mexican and Lisbon varieties respectively. The strong peak in the range of 1309 and 1303cm^{-1} suggests the stretching vibration of alcohols, carboxylic acid and esters. Comparable study by has showed IR peaks at 4000-600 cm^{-1} for a sample of pectin present as a drug mixture (Kanmani *et al*., 2014).

Aromatic ethers show a strong band near 1250 cm^{-1}, while cyclic ethers show a C–O stretching band over a broad 1250–900 cm^{-1} range. Aliphatic and aromatic ketones show carbonyl bands at 1730–1700 and 1700–1680 cm^{-1}, respectively, while aliphatic and aromatic aldehydes produce

carbonyl bands in the 1740–1720 cm^{-1} and 1720–1680 cm^{-1} ranges, respectively (see Table 4.7). Aldehydes also show a characteristic C–H stretching band in the 2900–2700 cm^{-1} range.

Aromatic and aliphatic esters may be differentiated, as both C=O stretching and C–O stretching vibrations produce bands in different ranges: aliphatic esters produce C=O and C–O bands at 1750–1730 and 1300–1100 cm^{-1}, respectively, while aromatic esters produce C=O and C–O bands at 1730–1705 and 1310–1250 cm^{-1}, respectively (Stuart, 2004).

Table 4.7 functional groups present in Mexican and Lisbon varieties

Bond	Functional group	Frequencies (cm^{-1}) Mexican variety	Frequencies (cm^{-1}) Lisbon variety
O-H stretch, H-bonded	Phenols	3447	3441
C-H stretch	Alkanes	3057	3040
C-H stretch	Aldehyde	2936	2937
C-H stretch	Aldehyde	2857.5	2849
C=O stretch	Aliphatic, ketone	1700	1760
C=C stretch	Alkenes	1655	1648.5
C-O stretch	Aliphatic, Esters	1211.5	1210.5
C=O stretch	Anhydride	1749	1760
C-O stretch	Aromatic, Esters	1309	1303

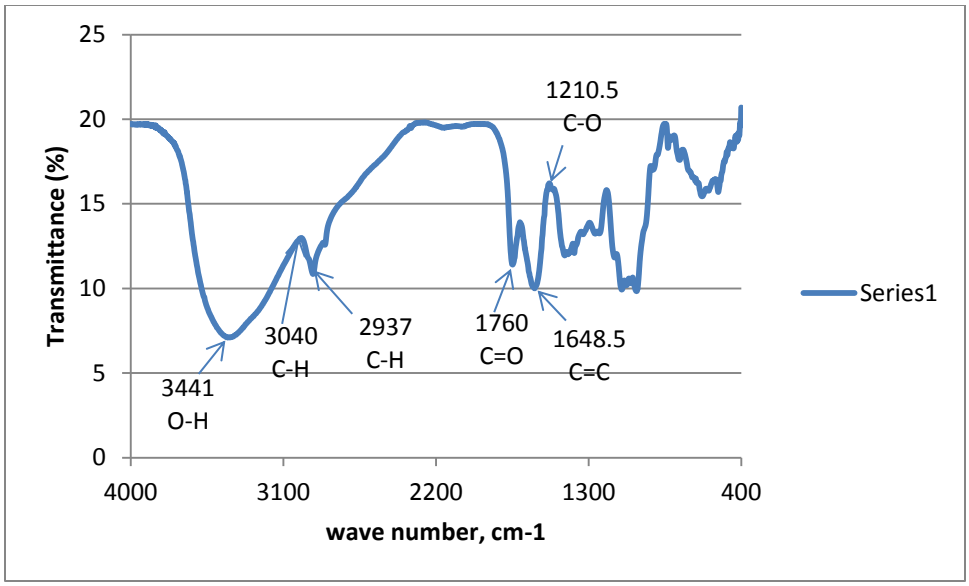


Figure 4-15 FTIR spectra of pectin extracted from Lisbon

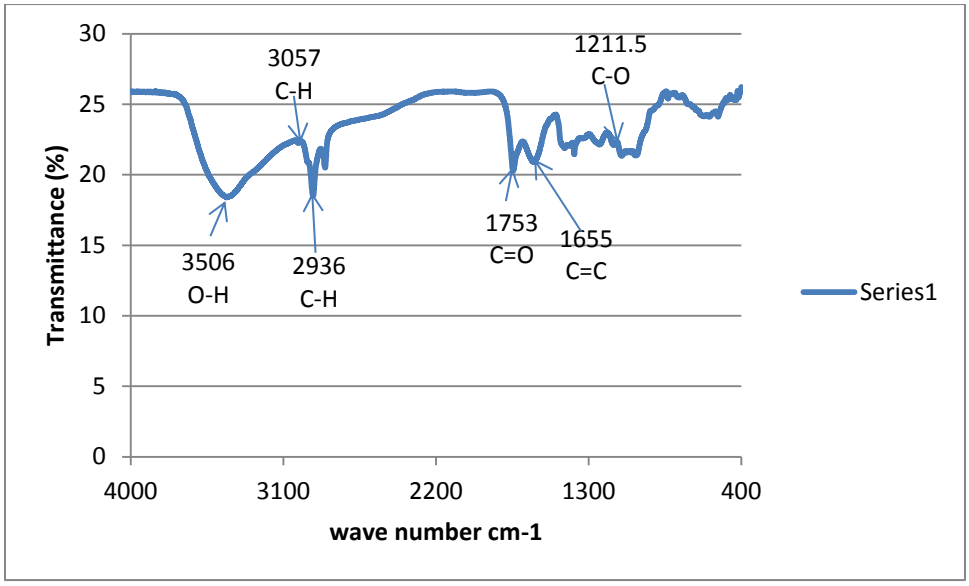


Figure 4-16 FTIR spectra of pectin extracted from Mexican variety

5. SUGGESTED PROCESS TECHNOLOGY FOR EXTRACTION OF PECTIN

5.1 Process description for pectin production

Pectin is produced from citrus fruits and apple pomace. Research samples collected from Upper Awash Agro- Industry Enterprise were milled using centrifugal mill in the mechanical unite operation laboratory and sized in the required size (70 MESH). The powdered peel is subjected to further extraction using conical flask of 250 ml. distilled water of 150ml, 5g lemon powder, and adding 1M HNO_3 acid to maintain the pH of the solution at the desired pH of 1,2.5, and 4. The water bath was set to desired temperature of 40, 60, and 80 °C and kept for two hours for the extraction of pectin from the peel. After two hours of extraction the extract was separated using centrifugal machine which runs at 3000 rpm for 15 min. The extract is easily separated and the clear solution is treated with equal amount of 96% alcohol for two hours. The coagulated pectin if skimmed off and dried at 50 °C for 24 h, finally the dried pectin is milled and packed in air tight poly ethylene bag for further use. From the experiments that have been conducted maximum pectin yield was observed at temperature 60 °C and pH 1.

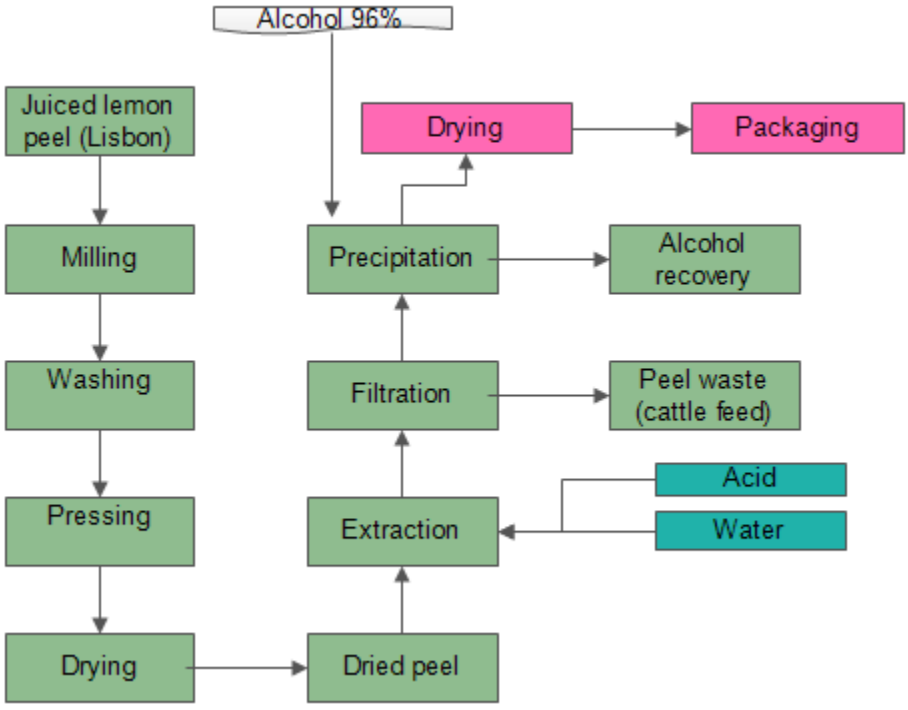


Figure 5-1 Basic steps production of pectin from lemon peel

Basic operating methods

Given the choice made for the basic production process of pectin from lemon peels, the next decision will be how the plant will actually operate.

Batch operation – Batch operations are conducted in discreet units. Each batch of feedstock is processed independently of the next for all processes such as preparation, reaction, separation, purification, and drying. These type operations can be easily started and stopped and are good for facilities that do not operate 24 hours a day. The product flow can be easily traced and operations are of the batch variety.

Continuous operations – As the name implies, the process is an on-going flow of feed stocks, preparation, reactions and separations. These type facilities are typically the largest and most efficient operations. They often can get by with lower inventories and thus less storage space. These processes rely upon a consistent feedstock, typically single sourced, and are sensitive to process changes.

Batch-continuous – This approach attempts to reap the benefits of both of the above processes as it adapts characteristics of both. In practice it typically consists of batch reactors making independent batches of product followed by a continuous separation and purification process. It garners the efficiencies at the back end of the process while enjoying the ability to control the extraction process and change feed stocks more rapidly than the continuous systems. The following table compares characteristics of the batch with the continuous systems.

Table 5.1Comparison of pectin production technology

Characteristics	Batch	Continuous
Typical capital cost	Less	Greater
Economy of scale	< 500 tons per year	>500 tons per year
Feedstock flexibility	Greater flexibility	Less flexibility
Consumption of input	Greater	Less
Operating cost per kilogram	Greater	Less
Automation	Less	Greater
Product yield	Less	Greater
Product quality consistency	Less	Greater
Typical plant size	Smaller	Larger

(Source: Coulson and Richardison 4^{th} vol.6)

From the alternatives given in Table (5.1), a continuous process is selected to be compatible with the estimated plant capacity for pectin production.

Material and energy balance

5.2 Material balance

The capacity of pectin production plant is determined based on market study of marmalade producing companies in Ethiopia the total pectin consumption in Ethiopia is around 494 kg per day.

This feasibility study is done assuming Merti-Agro Processing Industry produces lemon juice and the waste (lemon peel) is going to be used for production of pectin. Assuming 270 working days per year, pectin consumption per year = 148.148kg/year x 270 day/year =40,000 kg/year. for plant cost estimation and cost analysis taking 30% of the market demand.

Basis: 1day
From the literature review and laboratory results, the amount of all the raw material needed and amount of product and byproduct from the plant for the production of pectin is calculated as shown below.

5.2.1 Material balance

Material balances had been performed based on the experimental work in the laboratory. The material balance will enable us to quantify the substances going in and out of the entire process and piece of equipment's. The amount of losses that were expected in commercial production of pectin is also determined. Nearly all fruit processing result in loss of material. These arise from peeling, unsatisfactory fruits rejected during sorting, spillage during filling into packs and from food stick on equipment and lost during washing. Process losses in each major unit operations were estimated based on process loss in a well-managed fruit and vegetable processing plant

Table 5.2 Typical losses during processing of fruits and vegetables

Stages in a process	Typical losses
Washing fruits/ vegetables	0-10
Sorting	5-50
Peeling	5-60
Slicing/dicing	5-10
Boiling	5-10
Drying	10-20
Packaging	5-10

(Source: Fellows, P., Midway Technology Ltd, Bonsall, UK)

Laboratory test result:

From laboratory experiments conducted the Lisbon variety has highest percentage yield of 21%. Four samples were taken randomly and measured with peel and without peel to see which variety have highest peel to juice ratio. Table below shows that the Lisbon variety has maximum peel to fruit ratio of 31.8% and the Mexican variety has 14% peel ratio. This figure makes it easier to choose the Lisbon variety for production purpose.

Table 5.3 Random samples and their weight

	Mexican whole fruit(g)	Mexican only peel(g)	Lisbon whole fruit(g)	Lisbon only fruit(g)
1	53.78	7.30	149.09	50.95
2	34.33	5.78	170.37	55.67
3	49.48	5.57	122.15	32.25
4	36.405	5.62	162	53.36
Total	173.995	24.27	603.61	192.23
Average	43.5	6.0675	150.9025	48.0575

The laboratory data for extraction of pectin from lemon peel provides information useful for material balance. Material balance as per laboratory scale is summarized as follows:

Mass of fresh lemon peel = 3.3148kg

Mass of dried lemon peel = 0.663kg

Mass of lemon dried peel powder = 0.597kg

Mass of dried pectin produced = 0.5705kg

Mass of pectin powdered = 0.165kg

Ratio of lemon peel powder to distilled water (1:30), w/v%

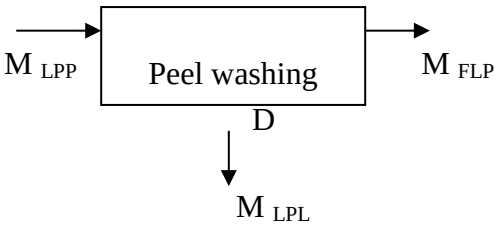
Product commercialization (Scale up the laboratory scale lemon peel pectin process into small scale processing industry)

The process selected for the manufacture of pectin is a continuous process. The laboratory result shows that one can get an output of 0.165 kg of pectin from 3.3148kg of lemon peel. To scale up this in to agro-processing process industry with overall assumptions of 148.148 kg production of pectin per day, we need 2,962.245 kg lemon peel supply per day. The plant operates 270 days per year, so the annual production of pectin will be: 148.148kg/day * 270days per year = 39,999.96kg = 40ton per year.

Since the intended daily production of pectin is 148.148kg, we can take out a scale up factor for multiplying all the laboratory scale results to obtain the daily needs of all the inputs and outputs of the whole process. This can be done by dividing 148.148kg of pectin/day with 0.165kg of pectin. Therefore, we get a scale up factor of 894.07 for daily production of pectin. Therefore, based on the factor material balance is presented below.

Material balance on the peel washing:

The received Lemon peel was washed in washing tank. Assume 5% of lemon peels loss during washing.



From overall material balance:

Where,

M_{LPP} = Fresh lemon peel to be processed

M_{LPL} = lemon peel loss

M_{FLP} = Mass of fresh washed lemon peel

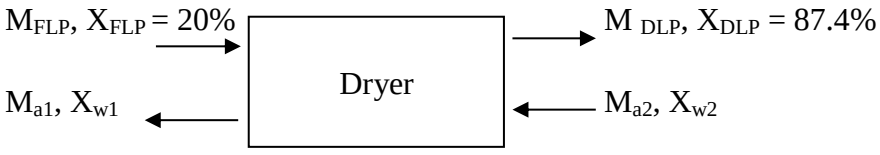
Input = Output

$M_{LPP} = M_{MPL} + M_{FLP}$

M_{FLP} = 2,962.245kg/day – 0.05*2,962.245kg/day = 2,814.132 kg/day

Material balance on the lemon peel dryer:

The major component wet fruit peel wastes are approximately 80% water, 6% soluble sugars, 5% cellulose and hemicelluloses, 4% pectin and 0.8% limonene (Welyang Z et al., 2008). The flow of the drying material and hot air is counter- current.



Where,

M_{FLP} = Mass of fresh lemon Peel

M_{DLP} = Mass of dry lemon peel

M_{a1} and M_{a2} – drying air flow rates at the outlet and inlet, respectively

X_{w1} and X_{w2} – moisture content of air at inlet and outlet, respectively

X_{FLP} = Mass fraction of solid in fresh lemon peel

X_{DLP} = Mass fraction of dry lemon peel

T_{a1} and T_{a2} – temperature of air at the outlet and inlet, respectively

T_{FLP} and T_{DLP} – temperature of lemon peel at the inlet and outlet of the dryer, respectively

Assumptions:

- Steady state flow of drying gas and lemon peel (ma1 = ma2 = ma)
  T_{FLP} = 25 °C and T_{DLP} = 40 °C
- Inlet air (T_{a2} = 60 °C and relative humidity RH = 10%) – from the psychometric chart
  X_{W2} = 0.014 kg H_2O/kg solid;
  Outlet air, T_{a1} = 35 °C, reference temperature T_0 = 0 °C;
- At a pressure of 101,325 Pa, latent heat of vaporization of water, λ = 2257 kJ/kg

From overall material balance:

In put = Out put

Component material balance on the Drier:

$$M_{FLP} * X_{FLP} + M_{a2} * X_{w2} = M_{DLP} * X_{DLP} + M_{a1} * X_{w1}$$

$$= 2{,}814.132\text{kg/day} * 0.2 + M_{a1} * 0.014 = M_{DLP} * 0.874 + M_{a1} * X_{w1}$$

$$M_{DLP} = \frac{562.82 - 0.986Ma}{0.874} \quad \text{equation (*)}$$

Energy Balance

$$M_{FLP} * H_{FLP} + M_{a2} * H_{a2} = M_{DLP} * H_{DLP} + M_{a1} * H_{a1} + q$$

Where:

q – thermal energy loss of the dryer

H_a – thermal energy content of air (kJ/kg dry air)

H_{FLP} &H_{DLP} – thermal energy content of lemon peel before drying and after drying (kJ/kg dry peel)

Assuming an adiabatic system, the above equation can be rewritten as:

$M_{FLP} * H_{FLP} + M_{a2} * H_{a2} = M_{DLP} * H_{DLP} + M_{a1} * H_{a1}$

Thermal energy content of air can be expressed as:

$Ha = Cs\ (Ta - To) + W*\lambda$

Cs is humid heat of air, $Cs = 1.005 + 1.88W$

Where, Ta– air temperature (°C) Substituting $Ha = (1.005 + 1.88W)\ (Ta - To) + X_w*\lambda$

$H_{a1} = 1.005 + 1.88X_{W1}\ (35 - 0) + 2256.9\ X_{W1}$, and

$H_{a2} = 1.005 + 1.88X_{W2}\ (60 - 0) + 2256.9\ X_{W2}$

H_{a1} = 2357.975 KJ/kg of dry air

H_{a2} = 93.47 KJ/kg of dry air

$H_{FLP} = Cpp(T_{FLP}\text{-}To) + X_{FLP} * Cp,w(T_{FLP}\text{-}To)$

$= 1.907(25\text{-}0) + 0.2 * 4.187(25\text{-}0)$

= 81.17 KJ/kg

$H_{DLP} = Cp,p\ (T_{DLP}\ \text{-}Tref) + X_{DLP} * Cp,w\ (T_{DLP}\ \text{-}Tref)$

$= 1.907(60\text{-}0) + 0.874 * 4.187(60\text{-}0)$

= 333.98 KJ/kg

$M_{FLP} * H_{FLP} + M_{a2} * H_{a2} = M_{DLP} * H_{DLP} + M_{a1} * H_{a1}$

$2814.132 * 81.171 + M_{a2} * 93.47 = M_{DLP} * 333.98 + M_{a1} * 2357.97$

$2814.132 * 81.171 + M_{a2} * 93.47 = M_{DLP} * 333.98 + M_{a1} * 2357.97$, since $M_{a1} = M_{a2}$

$228425.9 = M_{DLP} * 333.98 + Ma * 2354.5$ equation (**)

Substitute equation (*) into (**) and solving for both Ma and M_{DLP}

Ma = 6.75 kg/day, and M_{DLP} = 643.96kg/day

Summary of material and energy balance around the drier

Mass flow rate of water entering with the wet lemon peel

= 2814.132kg lemon peel/h*0.8kg H_2O/kg lemon peel

= 2251.3 kg/day

Mass flow rate of dried lemon peel (zero moisture content)

= 2814.13- 2251.3 = 562.832kg/day

Mass flow rate of dried lemon peel leaving with 12.6% moisture content

Mp = 562.83 + (0.126*562.83) = 633.74kg/day

Mass of evaporated water

M_{w2} = (2251.3-70.91) = 2180.38kg/day

Heat load calculation

Heat leaving the drier with the dried lemon peel

Qp = Mp*Cp,p*T = 633.74*1.907*(40-25) = 18128.13kJ/h

Heat leaving the drier with water vapor in the drying air

$Q_w = M_{w2}*\lambda + M_{w2}*C_{pa} *\Delta Ta$

= (2180.38*2257) + (2180.38*4.187)*(60-35)

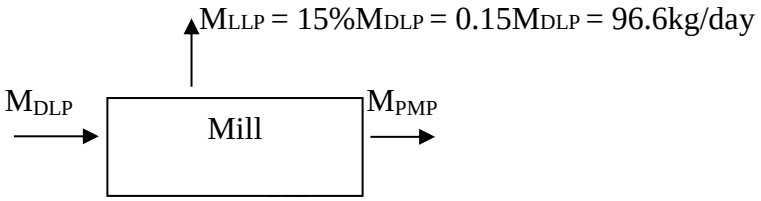
Q_{w2}= 5149168.37 KJ/h

Total heat leaving the drier

Q= Qp + Qw = 5150980.5 KJ/h

Material balances on dried lemon peel milling:

The dried lemon peel was milled by a centrifugal mill. From Table 5.2 the average loss associated with milling was estimated as 15%.



Where,

M_{DLP} = Mass of dry lemon peel

M_{LLP} = Loss of dry lemon peel

M_{PLP} = Powder of dry lemon peel

From overall material balance:

Input = Output

M_{LLP} = 15%M_{DLP} = 0.15M_{DLP} = 0.15* 643.96=96.6kg/day

M_{DLP} = M_{LLP} + M_{PLP} *M_{PLP} = M_{DLP} - M_{LLP} = 643.96kg/day – 96.6kg/day

= 547.366kg/day

Material balance on blender:

It is the equipment used to make homogenous suspension of distilled water, nitric acid and dry fruit peel powder. The experimental result revealed that for the maximum pectin yield the ratio of powder dry lemon peel, distilled water and 1 M HNO_3 (1g: 30ml: 8.4ml). Density of acid 1.42g/cm^3 and density of water 1g/ml.

Maas of 1MHNO_3, m = ρ * ν, Where, ρ = Density of 1MHNO_3

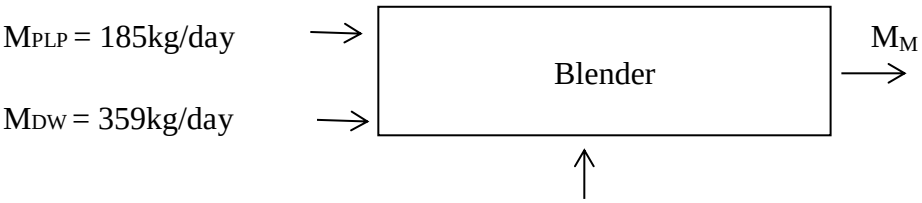
V = Volume of 1MHNO_3

m = ρ * ν = 1.42g/ml * 145.717 ml = 207g =0.207kg * 894.07 = 185kg/day

Mass of distilled water, m = ρ * V, Where, ρ = density of water

V = volume of distilled water

m = ρ * V = 1g/ml * 401.64ml = 401.64g = 0.4016kg * 894.07 = 359.093kg/day



Where,

M_{SA} = Mass of nitric acid

M_{DW} = Mass of distilled water

M_M = Mass of Mixture

M_{PLP} = Mass of dry lemon peel powder

Overall material balance:

In put = Out put

$M_M = M_{NA} + M_{DW} + M_{PLP}$

= 185kg/day + 359kg/day + 547.36kg/day = 1,091.46kg/day

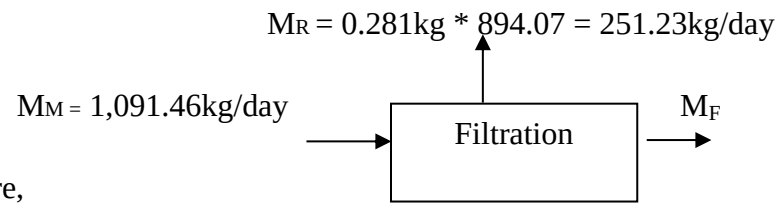
From overall material balance

In put = Out put

$M_M = M_{EM}$ = 1,091.46kg/day

Material balance on filtration:

The filtration process was carried out by a filter with cloth. The experimental result showed that for the maximum pectin yield of the mass of extract after filtration was found to be 0.281kg.



Where,

M_M = Mass of mixture

M_F = Mass of filtrate

M_R = Mass of residue

From overall material balance

Input = Output

$M_M = M_F + M_R$

$M_F = M_M - M_R$ = 1,091.46kg/day – 251.23kg/day = 840.23kg/day

Material balance on 96% ethanol blending:

An equal volume of 96% ethanol was used for the concentrated lemon peel pectin extract for the purpose of precipitation.

Where,

M_{LPE} = Mass of lemon peel extract- ethanol solution

M_E = Mass of 96% ethanol

From overall material balance:

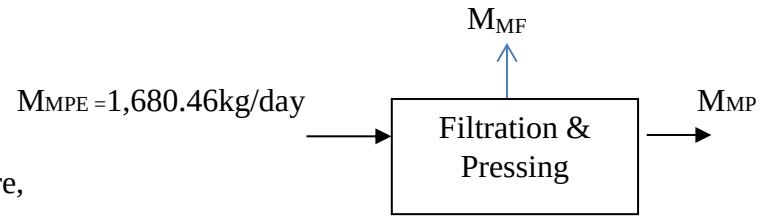
Input = Output

$M_E + M_F = M_{LPE}$

M_{MPE} = 840.23kg/day +840.23kg/day = 1,680.46kg/day

Material balance on filtration and pressing:

The result of the experimental trial showed that the filtered and pressed precipitate for lemon peel at the maximum pectin yield it contained mass filtrate to be 0.6427kg or 574.61kg/day.



Where,

M_{MP} = Mass of precipitate

M_{MF} = Mass of filtrate

From overall material balance

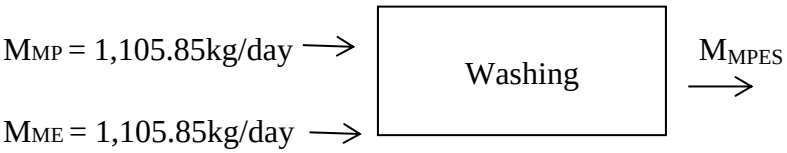
Input = Output

$M_{MPE} = M_{MF} + M_{MP}$

$M_{MP} = M_{MPE} - M_{MF}$ = 1,680.46kg/day – 574.61kg/day = 1,105.85kg/day

Material balance on 70% ethanol washing:

The precipitate was washed with an equal weight of 70% ethanol.



Where,

M_{ME} = Mass of 70% ethanol

M_{MPES} = mass of precipitate- ethanol solution

From overall material balance

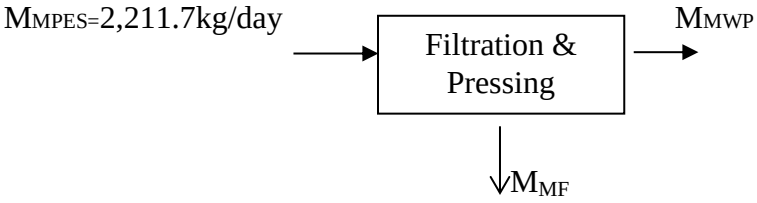
In put = Out put

$M_{MP} + M_{ME} = M_{MPES}$

$$M_{MPES} = M_{MP} + M_{ME} = 1{,}105.85\text{kg/day} + 1{,}105.85\text{kg/day} = 2{,}211.7\text{ kg/day}$$

Material balance on filtration and pressing:

The washed precipitate was once again filtered and pressed using a filtered. The result of the experimental trial showed that the filtered and pressed precipitate for lemon peel at the maximum pectin yield it contained mass filtrate 1.836kg or 1,641.51kg/day.



Where,

M_{MWP} = Mass of washed Precipitate

M_{MF} = Mass of filtrate

From overall material balance:

In put = Out put

$$M_{MPES} = M_{MF} + M_{MWP}$$

$$M_{MWP} = M_{MPES} - M_{MF} = 2{,}211.7\text{kg/day} - 1{,}641.51\text{kg/day} = 569.5\text{kg/day}$$

Material balance on the pectin dryer:

It is used to dry the filtered and pressed precipitate i.e. to make agreeable for easily grinding. The filtered and pressed precipitate was dried in oven at 60 ◦C to a constant weight. The flow of the drying material and hot air is counter - current.

Where,

M_{MDP} = Mass of dried precipitate

X_{MWP} = Mass fraction of washed precipitate

X_{MDP} = Mass fraction of dried precipitate

M_{a1} and M_{a2} – drying air flow rates at the outlet and inlet, respectively

X_{w1} and X_{w2} – moisture content of air at inlet and outlet, respectively

X_{MWP} = Mass fraction of solid in fresh lemon peel

X_{DP} = Mass fraction of dry pectin

T_{a1} and T_{a2} – temperature of air at the outlet and inlet, respectively

T_{MWP} and T_{DP} – temperature of lemon pectin at the inlet and outlet of the dryer, respectively

Assumptions:

- Steady state flow of drying gas and lemon peel (ma1 = ma2 = ma) T_{MWP} = 25 °C and T_{DP} = 40 °C
- Inlet air (T_{a2} = 60 °C and relative humidity RH = 10%) – from the psychometric chart X_{W2} = 0.014 kg H_2O/kg solid;
- Outlet air, T_{a1} = 35 °C, reference temperature T_0 = 0 °C;
  At a pressure of 101,325 Pa, latent heat of vaporization of water, λ = 2257 kJ/kg

From overall material balance:

In put = Out put

Component material balance on the Drier:

$$M_{MWP} * X_{MWP} + M_{a2} * X_{w2} = M_{DP} * X_{DP} + M_{a1} * X_{w1}$$

$$= 569.5\text{kg/day} * 0.2974 + M_{a1} * 0.014 = M_{DLP} * 0.874 + M_{a1} * X_{w1}$$

$$M_{DLP} = \frac{169.37 - 0.986Ma}{0.898} \quad \text{equation (*)}$$

Energy Balance

$M_{MWP} * H_{MWP} + M_{a2} * H_{a2} = M_{DP} * H_{DP} + M_{a1} * H_{a1} + q$

Where:

q – Thermal energy loss of the dryer

H_a – thermal energy content of air (kJ/kg dry air)

H_{MWP} &H_{DP} – thermal energy content of lemon pectin before drying and after drying (kJ/kg dry peel)

Assuming an adiabatic system, the above equation can be rewritten as:

$M_{MWP} * H_{MWP} + M_{a2} * H_{a2} = M_{DP} * H_{DP} + M_{a1} * H_{a1}$

Thermal energy content of air can be expressed as:

$Ha = Cs\ (Ta - To) + W\lambda$

Cs is humid heat of air, $Cs = 1.005 + 1.88W$

Where Ta– air temperature (°C) Substituting $Ha = (1.005 + 1.88W)\ (Ta - To) + X_w * \lambda$

$H_{a1} = 1.005 + 1.88X_{W1}\ (35 - 0) + 2256.9\ X_{W1}$, and

$H_{a2} = 1.005 + 1.88X_{W2}\ (60 - 0) + 2256.9\ X_{W2}$

H_{a1} = 2357.975 KJ/kg of dry air

H_{a2} = 93.47 KJ/kg of dry air

$H_{MWP} = Cp,p\ (T_{MWP}\text{-}To) + X_{FLP} * Cp,w\ (T_{MWP}\text{-}Tref)$

= 1.696(25-0) + 0.29*4.187(25-0)

= 93.47KJ/kg

$H_{DP} = Cp,p\ (T_{DLP}\text{ -}To) + X_{DP} * Cp,w\ (T_{DP}\text{ -}Tref)$

= 1.696(60-0) + 0.898* 4.187(60-0)

= 327.35 KJ/kg

$M_{FLP} * H_{FLP} + M_{a2} * H_{a2} = M_{DLP} * H_{DLP} + M_{a1} * H_{a1}$

$569.5 * 117.11 + M_{a2} * 93.47 = M_{DP} * 327.35 + M_{a1} * 2357.97$

$569.5 * 117.11 + M_{a2} * 93.47 = M_{DP} * 327.35 + M_{a1} * 2357.97$, since $M_{a1} = M_{a2}$

$66694.14 = M_{DP} * 327.35 + Ma * 2264.5$ equation (**)

Substitute equation (*) into (**) and solving for both Ma and M_{DLP}

Ma = 34.96 kg/day, and M_{DLP} = 155.24kg/day

Summary of material and energy balance around the drier

Mass flow rate of water entering with the washed precipitate

= 569.5kg lemon peel/day*0.7026kg H_2O/kg lemon peel

= 169.36 kg/day

Mass flow rate of dried lemon peel (zero moisture content)

= 569.5- 400.13 = 169.36kg/day

Mass flow rate of dried lemon peel leaving with 12.6% moisture content

Mp = 169.36 + (0.102*169.36) = 186.63kg/day

Mass of evaporated water

M_{w2} = (400.13-17.27) = 382.85kg/day

Heat load calculation:

Heat leaving the drier with the dried pectin

$Qp = Mp * Cp,p * \Delta T$ = 186.63*1.69*(40-25) = 4731.17kJ/day

Heat leaving the drier with water vapor in the drying air

$Qw = M_{w2} * \lambda + Mw2 * Cpa * \Delta Ta$

= 382.85*2257 + (382.85*4.187)(60-35)

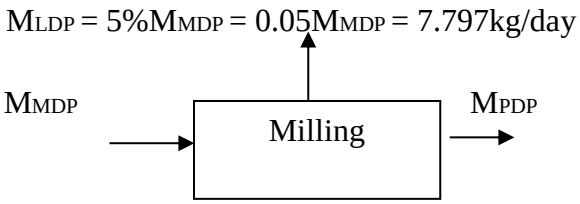
Qw= 904167.07 KJ/day

Total heat leaving the drier:

Q= Qp + Qw = 5150980.5KJ/day

Material balance on pectin milling:

The dried pectin was milled by a centrifugal mill. From Table 5.2 the average loss associated with milling was estimated as 15%.



Where,

M_{MDP} = Mass of dry pectin

M_{LDP} = Loss of dry pectin

M_{PDP} = Powder of dry pectin

From overall material balance:

Input = Output

$M_{MDP} = M_{LDP} + M_{PDP}$

$M_{PDP} = M_{MDP} - M_{LDP}$ = 155.945kg/day – 7.797kg/day = 148.148kg/day

5.3 Energy balance

The energy balance has been made to estimate the energy required for production of pectin. The energy is utilized in the form electrical energy. Electrical energy is used as a source of heat for the drying, milling and mixing. Therefore, the heat energy required for extraction of pectin is determined from energy balance equations. The heating rate (heat load) of a heat exchanger (Q kW), required to heat the product (Mi, kg) by a temperature difference (ΔT, K or °C), is estimated from the equation.

$$Q = M_{i*}C_{pi*}\Delta T \qquad (5.1)$$

Where, C_{pi} (kJ/kg K) is the specific heat of the product

Energy balance on lemon peels drying:

The energy required to dry the washed lemon peel can be obtained from energy balance on oven drying. The result of moisture analysis indicated that about 80% of lemon peel contains water while the remaining comprised lemon peel solids. The magnitude of specific heat values depends on the composition of the food. The relationships presented by Choi and Okos (1986) provide the basis for predicting specific heat magnitudes for food products. The following equation can be used for these predictions:

$C_p = \Sigma [C_{pi}\, m_i]$

Where,

C_{pi}= specific heat values for individual product components;

m_i = mass fractions for individual product components

The specific heat values for the individual food components depend on temperature as predicated by the relationships in Table 5.4.

Table 5.4 Specific heat relationships for food product components

Component	Temperature function
Protein	$Cp = 2.0082 + 1.2089 * 10^{-3} T - 1.3129 * 10^{-6} T^2$
Fat	$Cp = 1.9842 + 1.4733 * 10^{-3} T - 4.8008 * 10^{-6} T^2$
Carbohydrate	$Cp = 1.5488 + 1.9625 * 10^{-3} T - 5.9399 * 10^{-6} T^2$
Fiber	$Cp = 1.8459 + 1.8306 * 10^{-3} T - 4.6509 * 10^{-6} T^2$
Ash	$Cp = 1.0926 + 1.8896 * 10^{-3} T - 3.6817 * 10^{-6} T^2$
Water[a]	$Cp = 4.1289 - 5.3062 * 10^{-3} T + 9.9516 * 10^{-4} T^2$
Water[b]	$Cp = 4.1289 - 9.0864 * 10^{-5} T + 5.4731 * 10^{-6} T^2$
Ice	$Cp = 2.0623 + 6.0769 * 10^{-3} T$

Source: Choi and Okos (1986)

^{a} For the temperature range of – 40 °C to 0 °C

b For the temperature range of 0 °C to 150 °C

Therefore, based on the calculations within Table 5.2 and 5.3 the specific heat of the powder of lemon peel was found. C_p of Mixture at 60 °C = 1.907kJ/kg.°C

Mass of fresh lemon Peel (M_{FLP}) = 2,814.132kg/day

Assume temperature of fresh lemon peel (T_{ref}) = 20 °C

Temperature of dried fresh lemon peel out of oven (T_F) = 60 °C

Final moisture content of dried lemon peel = 7.96%

Latent heat of evaporation of water at 60 °C can be determined from the known value of ΔHV of water with corresponding critical temperature as:

$$\Delta HV\ (T2) = \Delta HV\ (T1)\ [\frac{T_c - T_2}{T_c - T_1}]^{0.38} \quad \text{.................................}(5.3)$$

Thus taking ΔHV (100 °C) = 2257kJ/kg with the corresponding value of Tc = 374.25 °C, Specific heat of water = 4.18kJ/kg.

Energy balance on dry lemon peel milling:

A centrifugal mill was used to reduce the size of the lemon and lime peel. The energy required for milling was calculated using bond’s law as

$$E = w\sqrt{\left(\frac{100}{d2}\right) -}\ w\sqrt{\left(\frac{100}{d2}\right)} \quad \text{..} \ (5.9)$$

Where, E (J) = energy required, W (J/kg) = bond work index (40,000-80,000 J/kg) for hard foods such as sugar or strain (Fellows, 2000)).

d_1 (m) = diameter of the feed material and

d_2 (m) = diameter of the ground material.

The result of the experimental trial showed that the average diameter of dry lemon and lime peel, d_1 = 1.6mm and d_2 = 0.177mm average diameter of lemon peel powder. Assuming that the dry lemon peel was harder than sugar the energy required for milling would be:

E = 40.132MJ/kg

Therefore, total energy required milling 643.96kg/day of the available dry lemon peel become:

E = 40.132MJ/kg *643.96kg/day = 25,843.4MJ/day

Energy balance on Mixer (blender):

The raw materials were mixed in a 250 ml conical flask and placed in a water bath at a temperature of 60 °C. This temperature was selected based on the design point corresponding to the maximum pectin yield. The heat supplied was the energy required to increase the temperature of the mixture solution from a room temperature (20 °C) to 60 °C.

Input energy = Output energy

$Q = M_p C_{PS} (T_F – T_{ref}) + M_{AW} CP_{AW} (T_F - T_{ref})$ …… (5.7)

Where,

M_M = Mass of mixture

T_{ref} and T_F = the initial and final temperature of the mixture of solution

M_p = mass of powder lemon peel

C_{PS} = specific heat of solid

M_{AW} = mass of acidified water = Mass of acid + mass of distilled water

C_{PAW} = specific heat of acidified water = 4.18kJ/kg K

To determine the specific heat capacity of the mixture, we use:

$Cp, mixture = \Sigma (Cp_i X_i)$

Where,

Cp_i = The specific heat capacities of each component in the mixture, and

X_i = Mass fractions of each components

The moisture content of lemon peel was 12.6% obtained from moisture content analysis (the remaining 87.4% is solid), the specific heat of solid calculated as:

$C_{PS} = C_{PW}X_W + C_{PS}X_S = 4.18*0.126 + 1.907*0.874 = 2.19kJ/kg\ K$

Where, C_{PW} = specific heat of water

X_W = mass fraction of water

C_{PS} = specific heat of solid

X_S = mass fraction of solid

$M_M = 359.093kg/day + 547.36kg/day + 185kg/day = 1,091.46kg/day$

From equation 5.7

$Q = M_P*C_{PS}\ (T_F - T_{ref}) + M_{AW}\ C_{PAW}\ (T_F - T_{ref})$

$= 547.36kg/day * 2.088kJ/kg\ K\ (60 - 20) + 544.093kg/day * 4.18\ kJ/kg\ K\ (60 - 20)$

$= 136,687.85kJ/day$

$= 136.687MJ/day$

5.4 Equipment sizing and selection

The equipment design for this preliminary process evaluation involves determining the size of the equipment in terms of the volume, flow per unit time, or surface area. The determination of the equipment capacity per hour basis is done with the general assumption of 270 working days per year with two shifts.

Washing tank selection:

Fruit peel washing tank: washing is proposed to be completed maximum within two hour, so:
Washer Capacity: 2,962.245kg/2h = 1,481.12 kg/h

Therefore, selected a fruit peel washer with capacity of washing 1,500kg of lemon peel per hour.

Storage tanks:

The volume of storage tanks is calculated from the material balance and respective density of materials as shown below.

Volume = m/ρ = $V_r = \frac{360kg/day}{1000kg/m^3} = 0.36m^3 = \frac{0.36}{0.9} = 0.4\ m^3$

Storage tank for HNO_3 solution one week operation (V02)

Density	1421Kg/m3
Temperature	
Construction of material	Rubber lined carbon steel
Capacity	

$V = \frac{185Kg/day}{1421Kg/m^3} = 0.13\ m^3 = \frac{0.13m^3}{0.9} = 0.15\ m^3$

Drying unit equipment selection:

Dryer Capacity: drying is also intended to be completed within maximum of four hours; so, 2,814.132kg/6h = 469kg/h

Therefore, selected a fruit peel dryer with capacity of drying 480kg of lemon peel an hour is selected.

Drying unit (pectin drying):

Dryer Capacity: drying is also intended to be completed within maximum of four hours; so, 569.5kg/6h = 94.91kg/h

Therefore, selected a fruit peel dryer with capacity of drying 120kg of lemon peel an hour is selected.

Milling unit equipment selection:

Milling Machine: milling is also intended to be completed within maximum of one hours; so, 643.96kg/2h = 322kg/h

Therefore, select milling machine with a capacity of 350kg of dry lemon peel per hour.

Mixing unit equipment selection:

Mixer (Blending Machine) Capacity: the blending and mixing process together should also be completed within three hour; so, 1,091.46kg/2hr = 545.73kg/hr. Therefore, select a mixer with a capacity of mixing 600kg of ingredients per hour.

Filtration plate unit equipment selection:

Filtration Capacity: filtration is also intended to be completed within maximum of two hours; so, 1091.46kg/3h = 363.83kg/h

Therefore, select filtration machine with a capacity of 400kg of filtrate per hour.

5.5 Economic evaluation of the plant

5.5.1 Plant capacity and location

The annual production capacity of the plant is scheduled to be 40,000 kg per annum of lemon peel pectin. The production capacity is based on projected demand and availability of the lemon peel that could be captured from juice processing house. The production commence on 2 shift and 270 working days per year. The production program does not include Sundays and national and public holidays. It was also considered that the plant would conduct annual maintenance when the supply of lemon fruit peel is low.

5.5.1.1 Location of the processing plant

To select proper locations and sites, certain key requirements or criteria should be set, that would allow the assessment of a number of potential locations, and the rejection of those not fulfilling those requirements. The remaining alternatives are subject to a more in-depth qualitative and quantitative analysis of technical and financial criteria, including social, environmental and economic aspects of location and site selection.

The basic criteria for selection of the pectin processing plant location was minimizing the average production cost, including transport and amount of handling time which means that fresh lemon peel are more likely to be in good condition when they arrive at the processing unit within a short period of time. Beside the supply of good water, availability of raw material, availability of manpower, proximity to transport facilities and adequate market were taken into

consideration. The major market for pectin is Addis Ababa and other state towns. Hence the choice for locating the processing plant near raw materials supply and market proximity would depend on the annual quantity of raw materials, ingredients, packaging materials and final products that are transported between the two sites. As a result, Bole-Nura Era was selected for locating the processing plant. Locating the plant near Bole-Nura Era has additional benefit in reducing injury from handling and deterioration from changes during long transportation after processing.

5.5.2 Equipment and manpower requirements

5.5.2.1 Equipment requirement

The cost of equipment was estimated using scaling factors. If the cost of a piece of equipment or plant of size or capacity q_1 is C_1, then the cost of a similar piece of equipment or plant of size or capacity q_2 can be calculated from:

$C_2 = C_1 (q_1/q_2)^{n}$

Cost of equipment (new) = Cost of equipment (old) $*(\frac{capacity\ of\ new\ equipment}{capacity\ of\ old\ equipment})^{n}$

Where, the value of the exponent which is dimensionless and n depends on the type of equipment or plant and for Food stuffs, n is assumed to be 0.66 (Perry and Green, 1999). The purchasing costs of the equipment were updated by using the cost indices equation.

Table 5.5 Major cost of equipment and machineries for pectin extraction

Description	Capacity	Quantity	Unit cost(birr)	Total cost(birr)
Fruit peel washing tank with (agitator)	1,500 kg/hr	2	35,511.00	71,022
Storage tank	0.52m^3	3	5,008.00	15,024
Mixing	600 kg/hr	1	9,850.00	9,850.00
Mixer, jacketed & agitated	2m^3	1	111,300	111,300
Dryer drum and rotary	480kg/hr	1	16,143.00	16,143.00
Cooling device	2m^3	2	25,110.50	50,221
Centrifugal mill	350kg/hr	1	234,100	234,100
Dryer drum and rotary	120kg/hr	1	116,513	116,513
Filtering plate	400kg/hr	1	45,000	45,000

Precipitation tank (stainless steel)	$4m^3$	1	36,021.00	36,021.00
Packing	40,000 pcs	1	10 cents	4,000
Laboratory equipment (pH meter & analytical balances)		1	15,000.00	15,000.00
Total cost				724,194

Costs obtained from the internet (http://www.matche.com/EquipCost/Index.htm, retrieved on May, 15, 2016).

Table 5.6 Estimation of fixed capital investment based on delivered equipment cost

Direct cost	% FCI	Birr
Purchased equipment	100	724,194
Purchased equipment installation	39	282,435
Instrumentation and controls (installed)	13	94,145
Piping (installed)	31	224,500
Electrical (installed)	10	72,419
Building (including services)	29	210,016
Yard improvement	10	72,419
Service facilities (installed)	55	398,306
Land	6	43,451
Indirect cost		
Engineering and supervision	32	231,742
Construction and expenses	34	246,225
Total direct and indirect plant costs		
Contractors fee (about 5% of directly & Indirect plant cost)	18	130,355
Contingency (about 10% of direct & indirect plant cost)	36	260,709
Fixed capital investment (FCI)	FCI= (DC+IC)	2,990,916

Source: (Timmerhaus & peters , 1991, pp. 183-187)

Table 5.6 shows that percent of delivered equipment cost for solid fluid processing plant.

Working capital is an additional investment needed above the fixed capital to start up and operate the plant to the point in which income is earned. Working capital investment: 10 –20% of TCI. Assume 15%

Total capital investment:

= FCI + WC

= 0.15 TCI + FCI

= 3,518,724

5.7 Estimation of total manufacturing cost (TMC)

= Production cost + fixed charges + Plant overhead expenses

1. Direct production cost:

A) Raw material cost:

The plant will have 270 working days.

The major raw materials required for production of pectin is lemon peel. All the raw materials are locally available. The raw material requirements and the corresponding cost are shown in table (5.7). The total annual cost of raw materials is estimated to be Birr, 4,421,750.

Table 2.7 Estimation of total raw material cost

No	Raw material	Daily Consumption In (tons)	Annual Consumption in (tons)	Unite price Birr/kg	Cost per annual
1	Ethanol	0.22	59.4	22	1,306,800
2	Nitric acid	0.12	32.4	18	583,200
Total					1,890,000

B) Operating labor (OL):

Table 5.8 Shows total cost of operating labor

No	Job requirement	Req. no	Monthly salary In birr	Annual expenditure In birr
(i). Administration				
1	Plant manager	1	6,500	78,000
2	Secretary	1	1,500	18,000
3	Accountant	1	2,000	24,000
4	Sales person	1	2,000	24,000
5	Guard	2	1,200	14,000
6	Messenger	1	1,200	14,000
7	Cleaner	2	1,200	14,400
8	Driver	2	2,000	24,000
9	Store keeper	1	2,500	30,000
10	Sub- total			
(ii). Production				
1	Chemical end’	1	3,500	42,000
2	Operator	2	2,500	60,000
3	Unskilled laborer	3	1,200	43,200
4	Chemist	1	3,000	36,000
5	Electrician	1	3,000	36,000
6	Safety manager	1	2,000	24,000
7	Quality control head	1	2,500	30,000
	Sub- total			271,200
	Total	41		512,400

c) Direct supervisory and electric labor: (10-25% of OL)

Assume 15%

= 0.15 * OL

= 76,860

D) Utilities:

Utility cost

Amount of water required to cool the two reactors is 148.1kg/hr.

Density of water @20°c = 1000kg/m^3

m =148.1 kg/hr*24hr/day*270day/yr

= 959,767 kg/yr

$V = \frac{mass}{density}$

$V = \frac{959{,}767 kg/hr}{1000 kg/hr}$

= 960m^3/yr

Estimated water consumption for general services will be 100m^3/yr.

Total water consumption = 1,060 m^3/yr. Water cost 5 ETB/m^3

Annual cost = 1,060*5 ETB = 5,300 ETB/yr

Electricity cost = 130 kWh*24h/day*300day/yr = 936,000 kw/yr

= 936,000*0.75 cents/kw = 702,000 ETB

Total utility cost = 5,300 + 702,000 = 707,300

E) Maintenance (M): (2-10% of FCI)

Assume 6%

= 2,990,916*0.06 = 179,455

F) Operating supplies (OS): (10-20% of maintenance)

Assume 15%

= 0.15 * M

= 26,918

G) Laboratory charges: (10-20% of OL)

Assume 15%

= 0.15 * OL

= 76,860

DPC = (A+B+C+D+E+F+G) = 3,392,933

2. Fixed charges:

a) Depreciation: (10% of FCI for machinery)

= 299,091

b) Local taxes: (1-4% of FCI)

Assume 2%

= 172,154

c) Insurances: (0.4-1% of FCI)

Assume 1%

= 59,818

d) Rent: (8-12% of FCI)

Assume 9%

=269,182

Therefore total fixed charges = 800,245

But, Fixed charges = (10-20% of TPC)

Assume 10%

Therefore Total product cost = $\frac{800,245}{0.1}$

= 8,002,450

3. Plant overhead cost:

50-70% of (OL+OS+M)

Assume 60%

= 0.6 * (OL + OS + M) = 0.6*(512,400+77,469+516,463) = 718,773

General expenses (GE):

A) Administration cost: (40-60% of OL)

Assume 50 %

= 0.5 * OL = 256,200

B) Distribution and selling price: (2-5% FCI)

Assume 2 %

= 0.02 * FCI

= 59,818

C) Research and development cost: (2-5% of every sales birr)

Let's assume that 1kg of pectin = 140 ETB

Sales = 148.148 kg/day*270day/annual*140ETB/kg*1000kg/tone = 5,600,000 ETB/annual

= 0.02 * sales = 112,000 birr

Therefore general expenses (GE) = A + B + C = 428,018

Therefore manufacturing cost (MC):

= Production cost + fixed charges + Plant overhead expenses

= 4,911,911

Total production cost:

= MC + GE

= 5,339,929

Unit cost:

$$= \frac{TPC}{plant\ capacity}$$

$$= \frac{5{,}339{,}929}{40\ ton/yr} = 133{,}498.225 \text{ birr/ton} = 133.5 \text{ birr/kg}$$

5.8 Economic evaluation

Gross earnings:

The plant is working for say 270 days a year

Product produced per day 10 ton

Unite price = 133.5 birr/kg

Selling price = birr 165 ETB/kg

Total income = 40,000 kg/year*165 birr/kg

= 6,600,000birr/yr

Gross income (profit) =Total income – total product cost

= 6,600,000 – 5,339,929birr/yr

= 1,260,071 birr/yr

Depreciation (DP) = $\frac{FCI}{5} = \frac{2{,}990{,}916}{5}$ = 598,183 ETB

Tax = 35%

Income $tax_{1\text{-}5}$ = 35% (Gp - DP) = 0.35(1,260,071 – 598,183) = 231,660 ETB

Income $tax_{5\text{-}10}$ = 35% GP = 0.35*1,260,071 = 441,024 ETB

Net $profit_{1\text{-}5}$ = GP – DP – Income $tax_{1\text{-}5}$ = 1,260,071 – 598,183 – 231,660 ETB

= 430,228 ETB

Net $profit_{5\text{-}10}$ = GP – Income $tax_{5\text{-}10}$ = 1,260,071 – 441,024 = 819,047 ETB

5.8.1 Rate of return (ROR)

ROR before tax

ROI overall = $\frac{\text{Total GP} - \text{Total DP}}{\text{TCI} * \text{project life year}} * 100\%$

$= \frac{10(1{,}260{,}071) - 5(598{,}183)}{3{,}518{,}724 * 10} * 100\%$

=27.31 %

$$ROI_{1\text{-}5} = \frac{\text{Annual GP} - \text{Annual DP}}{\text{TCI}} * 100\%$$

$$= \frac{1{,}260{,}071 - 598{,}183}{3{,}518{,}724} * 100\%$$

= 18.81%

$$ROI_{5\text{-}10} = \frac{\text{Annual GP}}{\text{TCI}} * 100\%$$

$$= \frac{1{,}260{,}071}{3{,}518{,}724} * 100\%$$

= 36.03%

ROI after tax

$$\text{ROI overall} = \frac{\text{Total Net Profit}}{project\ life * \text{TCI}} * 100\% = \frac{5(\text{Net profit}1-5)+5(\text{Net profit}5-10)}{10 * TCI}$$

$$= \frac{5(430{,}228)+5(819{,}047)}{10 * 3{,}518{,}724}$$

= 17.75%

$$ROI_{1\text{-}5} = \frac{\text{Annual(Net profit}(1-5)}{TCI} * 100\% = \frac{430{,}228}{3{,}518{,}724} * 100\%$$

=12.22%

$$ROI_{5\text{-}10} = \frac{\text{Annual(Net profit}(5-10)}{TCI} * 100\% = \frac{819{,}047}{3{,}518{,}724} * 100\%$$

=23.27%

$$\text{Annual NPav} = \frac{5(Net\ \text{profit}1-5)+5(\text{Net profit}5-10)}{10\ years}$$

$$= \frac{5(430{,}228)+5(819{,}047)}{10\ years}$$

= 624,637

5.8.2 Payback period

Project life is assumed 10 years using straight line method

$$= \frac{FCI}{\text{Annual NPav} + \text{Annual } Dep.}$$

$$= \frac{2{,}990{,}916}{624{,}637 + 598{,}183}$$

= 2.5 years

Net present value at year 5 and year 10

Assume the discount factor is 10%

$$\text{NPV}_5 = \frac{\text{Net cash flow(NP5)}}{(1+r)^n} = \frac{430{,}228}{(1+0.1)^5}$$

= 267,137.74

$$\text{NPV}_{10} = \frac{\text{Net cash flow(NP10)}}{(1+r)^n} = \frac{819{,}047}{(1+0.1)^{10}}$$

= 315,778

CONCLUSION

In this research, production of pectin from locally available citrus varieties *Mexican and Lisbon* varieties has been investigated. The two processing conditions affecting the percentage of pectin yield are namely extraction temperature and pH has also been studied. The outputs of the experiment conducted have been analyzed by employing Design-Expert 7.0.0, three-level-two-factor general factorial design, through analysis of physicochemical characteristics.

Maximum extraction yield of pectin from lemon and lime fruit peel is found to be as follows: temperature of 60 ◦C, extraction time of 2 h and pH of 1.0. Under these optimal conditions, 21.2% and 19.6% of pectin was extracted from Lisbon and Mexican varieties respectively which are similar with work done by Yadav *et al.*, (2015) with percentage yield of pectin maximum for sweet lime (21.0%) and (19.1%) for papaya peel powder using hydrochloric acid at treatment combination of pH 2.0 at 85 °C for 60 min. The percentage yield was minimum (5.1%) in sweet lime and (4.0%) in papaya peel at treatment combination of pH 3.0at 85 °C for 60min. but in our case extraction temperature of 40°C and pH of 4.0 has brought minimum yields of 5.2% and 4.6% for Lisbon and Mexican varieties respectively. FTIR confirms the presence of various functional groups in extracted pectin. Results exhibited that, acid extraction is found to be a suitable technique to extract the pectin from two varieties peel. Based on the analysis of experimental results; it is found that the two process variables exhibited significant interaction effect on the yield of pectin. The characteristics of the product such as moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid, and degree of esterification is as same as is in the range of those reported in the literature.

The greatest extraction yields in pectin were obtained when highest acid strength and exposure of highest temperature was employed. By using the general factorial analysis, we can conclude that extraction temperature and pH significantly affects the yield of pectin. The pectin extracted from the peels of lemon fruit exhibited medium to high degree of esterification (50.00%-64.56%): whereas pectin from lime is low methoxyl pectin with degree of esterification varying from (27.62-30.33%).

From the preliminary feasibility study for 148.148 kg/day capacity pectin production plant Birr 3,518,724 million total capital investment (TCI) is required. The product cost was found to be 133.5 Birr/kg. The project was financially feasible with 17.75% rate of return on investment

(RORI) after tax, Birr 624,637 annual net present value (NPav), 2 year and 6 months payback period. Therefore, from experimental analysis and preliminary feasibility study we can conclude that pectin production using Lisbon variety peel is feasible. Finally it can be concluded that pectin from Lisbon variety has a great potential for substitution of imported pectin for food processing industries.

RECOMMENDATION

The yield of pectin significantly decreases with increase of maturation so any researcher in the future should consider the maturity vs yield interaction. The yield of the two varietys should be considered at different pH with different mineral acids at different maturity stages and also different exteraction conditions should be considered.

A 46.46% pectin yield from orange peel has been reported in literature. Therefore, in the process of orange oil and pectin extraction, it has been reccomended to first exteract oil using simple distillation and then isolate pectin with acid hydrolysis technique Shekhar and Harshal (2012). It is highly recommend to check the yield after the extraction of essentail oil.

The Lisbon variety has highest yield of 21% as compared to the Mexican (19.6%). It als has highest peel to fruit ratio of 31.8% and the Mexican has only 14%. Due to this two reasons using Lisbon lemons for commercial production of pectin is recommended in comparison to the Mexican lime.

Inovative and uptodate methods of extraction of pectin from citrus fruits and other fruits should be considered by other researchers.

The preliminary feasibility study suggests that pectin production from lemon peel is feasible with over all ROI after tax of 17.75 %, and payback period of 2.5 years. Therefore any government or private company could invest and enjoy the benefit.

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Appendices

Appendix A Design Expert Statistical Analysis Results of Pectin Yield

Table 3 Model adequacy measure for lime peel pectin

Standard deviation	0.222	R-Squared	0.9983
Mean	11.81	Adj R-Squared	0.9978
+C.V.%	1.84	Pred R-Squared	0.9972
PRESS	1.61	Adeq precision	142.503

Table 4 Sequential model sum of squares [type 1] for lime peel pectin

Sources	Sum of squares	Df	Mean square	F-value	P-value	
Mean vs total	3766.09	1	3766.09			
Linear vs total	555.83	4	138.96	276.98	<0.001	
2FI vs linear	10.76	4	2.69	177.92	<0.001	Suggested
Residual	0.27	18	0.015			
Total	4332.96	27	160.48			

‘Sequential model sum of squares [type 1]” select the highest order polynomial where the additional terms are significant and the model is not aliased.

Table A3 Lack of fit tests

Source	Sum of squares	Df	Mean Square	F Value	P- value Prob>F	
Linear	10.76	4	2.69		<0.001	
2 FI	0.000	0				Suggested
Pure error	0.27	18	0.015			

‘Lack of Fit Tests’ wants the selected model to have insignificant lack of fit.

Table A4 Model summery statistics

Source	Std Dev.	R–squared	Adjusted R – squared	Predicted R - squared	PRESS	
Linear	0.71	0.9805	0.9770	0.9707	16.62	
2FI	0.12	0.995	0.9993	0.9989	0.61	Suggested

‘Model summary statistics” Focus on the model maximizing the “ Adjusted R- squared” and the “ predicted R- squared”

ANOVA for Response Surface Quadratic Model

Table 5 Analysis of variance table [classical sum of squares-type11]--Mexican variety

Source	Sum of squares	Df	Mean square	F-Value	p-value prob > F	Remarks
Model	565.88	5	113.18	2399.44	< 0.0001	Significant
A	122.83	1	122.83	2604.06	< 0.0001	
B	201.47	1	201.47	4271.37	< 0.0001	
AB	10.05	1	10.05	213.00	< 0.0001	
A^2	230.72	1	230.72	4891.57	< 0.0001	
B^2	0.81	1	0.81	17.21	0.0005	Significant
Residuals	0.99	21	0.047			
Lack of fit	0.72	3	0.24	15.83	< 0.0001	Significant
Pure error	0.27	18	0.015			
Cor total	566.87	26				

Final equation in terms of coded factors for Mexican variety (categorical factors)

Yield = + 11.81- 4.68*A[1] + 4.13*A[2] + 3.22*B[1] + 0.25*B[2] – 0.99*A[1]B[1] + 0.27*A[2]B[1] - 0.11*A[1]B[2] - 0.023*A[2]B[2]

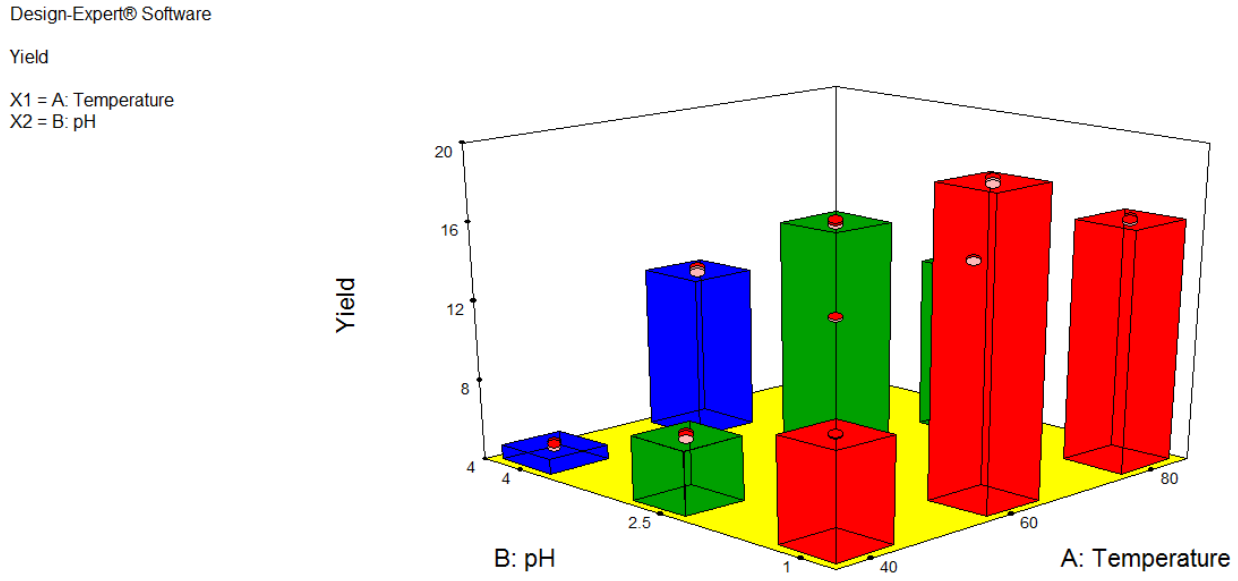

Figure A1 3D surface drawing showing effect of temperature and pH of Lisbon variety (Categorical factors)

Table 6 Coefficient estimate and confidence interval

Factor	Coefficient Estimate	Df	Standard Error	95% Cl	95% Cl	VIF
Intercept	16.19	1	0.093	16.00	16.38	1.00
A- Temperature	2.61	1	0.051	2.51	2.72	1.00
B- pH	-3.35	1	0.051	-3.45	-3.24	1.00
AB	-0.91	1	0.063	-1.05	-0.78	1.00
A^2	-6.20	1	0.089	-6.39	-6.02	1.00
B^2	-0.37	1	0.089	-0.55	-0.18	1.00

LISBON variety

Table A7 Model adequacy measure for Lisbon peel pectin

Standard deviation	0.75	R-Squared	0.9828
Mean	12.28	Adj R-Squared	0.9787
C.V.%	6.09	Pred R-Squared	0.9720
PRESS	19.09	Adeq precision	43.135

Table A8 Sequential model sum of squares [type 1] for Lisbon peel pectin

Sources	Sum of Squares	Df	Mean Square	F-value	P-value	
Mean vs total	4066.36	1	4068.36			
Linear vs mean	365.59	2	182.80	13.91	0.2226	
2FI vs Linear	20.18	1	20.18	1.57	0.2226	
Quadratic vs 2FI	283.56	2	141.78	253.74	<0.0001	Suggested
Cubic vs Quadra	11.22	2	5.61	207.70	<0.0001	Aliased
Residual	0.51	19	0.027			
Total	4749.43	27	175.90			

‘Sequential model sum of squares [type 1]” select the highest order polynomial where the additional terms are significant and the model is not aliased.

Table A9 Lack of fit tests for Lisbon peel pectin

Source	Sum of squares	Df	Mean Square	F Value	P- value Prob>F	
Linear	315.29	6	52.55	5323.88	<0.0001	
2 FI	295.12	5	59.02	5979.83	<0.0001	
Quadratic	11.56	3	3.85	390.28	<0.0001	Suggested
Cubic	0.34	1	0.34	34.00	<0.0001	Aliased
Pure error	0.16	18	0			

‘Lack of Fit Tests’ want the selected model to have insignificant lack of fit.

Table A10 Model summery statistics for Lisbon variety pectin

Source	Std Dev.	R–squared	Adjusted R - squared	Predicted R - squared	PRESS	
Linear	3.63	0.5368	0.4982	0.4308	387.65	
2FI	3.58	0.5664	0.5099	0.4368	383.61	
Quadratic	0.75	0.9828	0.9787	0.9720	19.09	Suggested
Cubic	0.16	0.9992	0.9990	0.9986	0.98	Aliased

‘Model summary statistics ‘Focus on the model maximizing the ‘Adjusted R- squared “ and the “ predicted R- squared”.

ANOVA for Response Surface Quadratic Model

Table A11 Analysis of variance table [classical sum of squares-type 11]- Lisbon variety

Source	Sum of squares	Df	Mean square	F-Value	p-value prob > F	Remarks
Model	669.33	5	133.87	239.15	< 0.0001	
A	171.68	1	171.68	307.25	< 0.0001	Significant
B	193.91	1	193.91	347.25	< 0.0001	
AB	20.18	1	20.18	36.11	< 0.0001	
A^2	281.03	1	281.03	502.95	< 0.0001	
B^2	2.53	1	2.53	4.52	< 0.0455	

Residuals	11.73	21	0.56			
Lack of fit	11.56	3	3.85	390.28	< 0.0001	Significant
Pure error	0.18	18				
Cor total	681.06	26				

Table A12 Coefficient estimate and confidence interval

Factor	Coefficient Estimate	Df	Standard Error	95% Cl	95% Cl	VIF
Intercept	17.27	1	0.32	16.60	17.94	1.00
A- Temperature	3.09	1	0.18	2.72	3.45	1.00
B- pH	-3.28	1	0.18	-3.65	-2.92	1.00
AB	-1.30	1	0.22	-1.75	-0.85	1.00
A^2	-6.84	1	0.31	-7.48	-6.21	1.00
B^2	-0.65	1	0.31	-1.28	0.014	1.00

Final equation in terms of coded factors for Lisbon variety (categorical factors)

Yield = +12.28-5.37*A[1] + 4.56*A[2] + 3.07*B[1] + 0.43*B[2] -1.86*A[1]B[1] + 1.00* A[2]B[1] + 0.029*A[1]B[2] + 0.22*A[2]B[2]

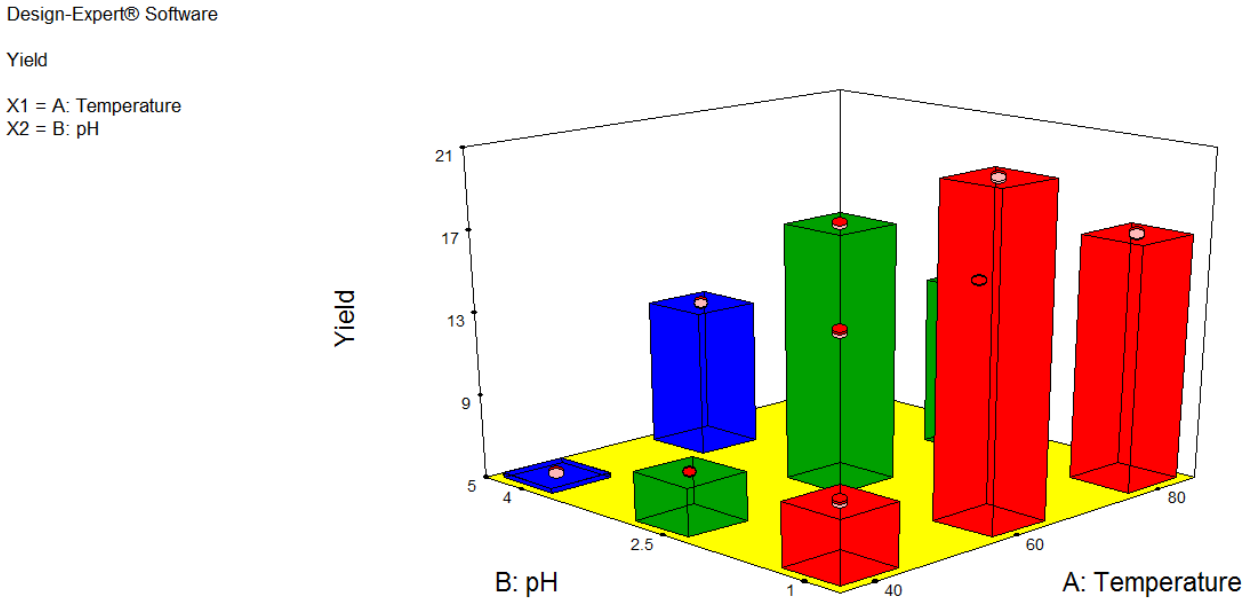

Figure A2 3D surface drawing showing effect of temperature and pH of Lisbon variety (Categorical factors)

Table A13 Shows laboratory result (yield) of Lisbon variety

Run	Temperature °C	pH	% Yield
1	40	1	7.97
2	40	1	8.11
3	40	1	8.24
4	60	1	20.8
5	60	1	21
6	60	1	20.9
7	80	1	17.14
8	80	1	16.91
9	80	1	17
10	40	2.5	7.4
11	40	2.5	7.31
12	40	2.5	7.39
13	60	2.5	17.5
14	60	2.5	17.42

15	60	2.5	17.56
16	80	2.5	13.2
17	80	2.5	13.32
18	80	2.5	13.27
19	40	4	5.32
20	40	4	5.21
21	40	4	5.2
22	60	4	12.22
23	60	4	12.04
24	60	4	12.1
25	80	4	8.8
26	80	4	9
27	80	4	9.1

Table A14 Shows laboratory result (yield) of Mexican variety

Run	Temperature °C	pH	% Yield
1	40	1	9.4
2	40	1	9.3
3	40	1	9.4
4	60	1	19.6
5	60	1	19.4
6	60	1	19.3
7	80	1	16.4
8	80	1	16.2
9	80	1	16.3
10	40	2.5	7.4
11	40	2.5	7.3
12	40	2.5	7.1
13	60	2.5	16
14	60	2.5	16.2

15	60	2.5	16.3
16	80	2.5	12.8
17	80	2.5	12.7
18	80	2.5	12.7
19	40	4	4.6
20	40	4	4.9
21	40	4	4.78
22	60	4	12.4
23	60	4	12.2
24	60	4	12.1
25	80	4	8
26	80	4	8
27	80	4	8.1

Appendix B: Lab experimental works

Figure B1-1 Lisbon variety collected from UAAIE

Figure B1-2 Mexican variety collected from UAAIE

Figure B1-3 The peel and juice being separated in juice extractor

Figure B1-4 Dried peels of Mexican variety (left) and Lisbon variety (right)

Figure B1-5 At left Lisbon variety and at right Mexican variety peel powder

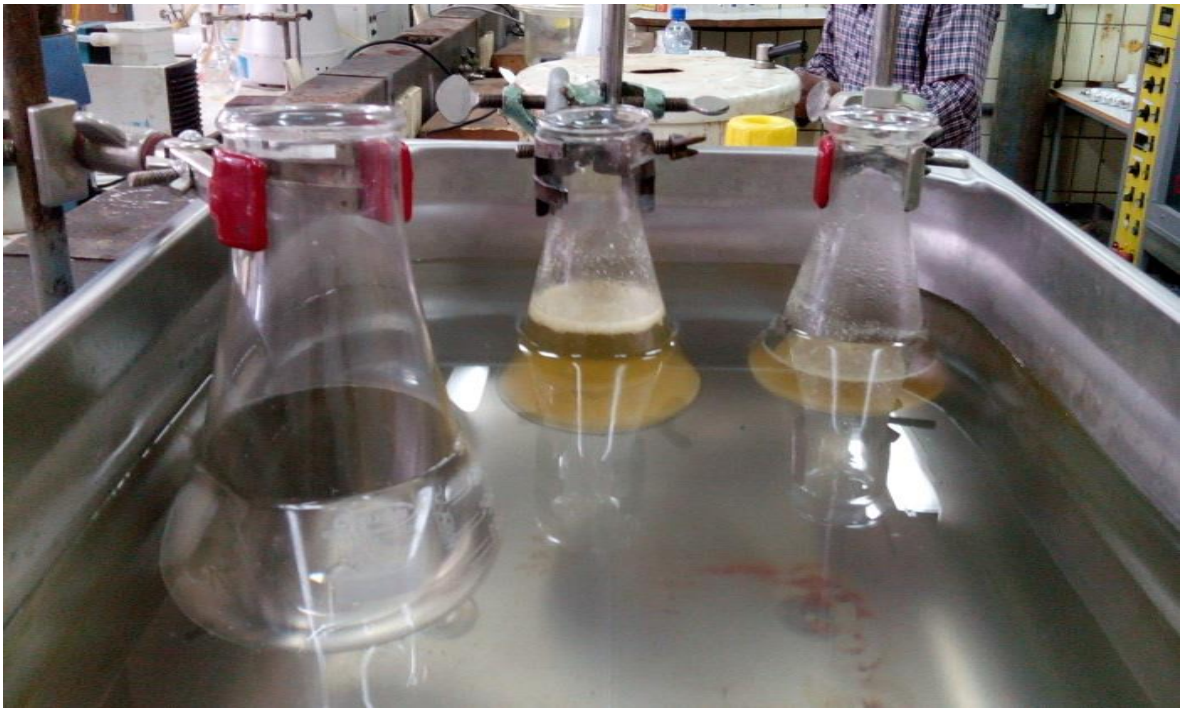

Figure B1-6 Extraction at water bath

Figure B1-7 At left extraction with residues and at the right clear solution from centrifuge

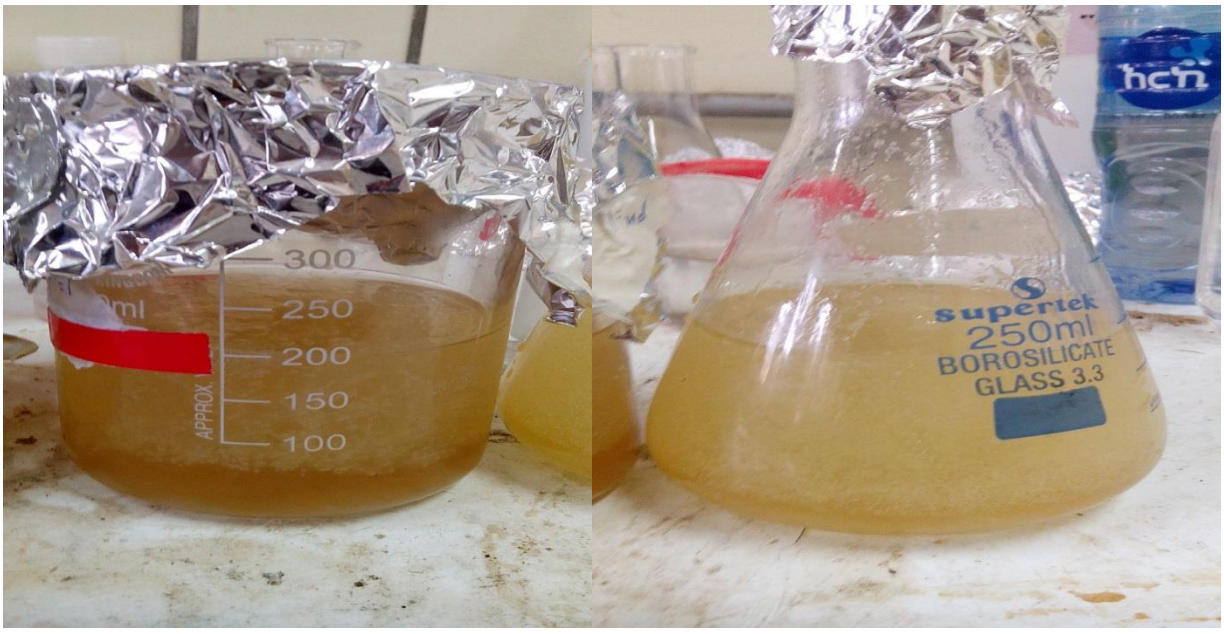

Figure B1-8 After addition of 96% ethanol on the clear solution and coagulated pectin

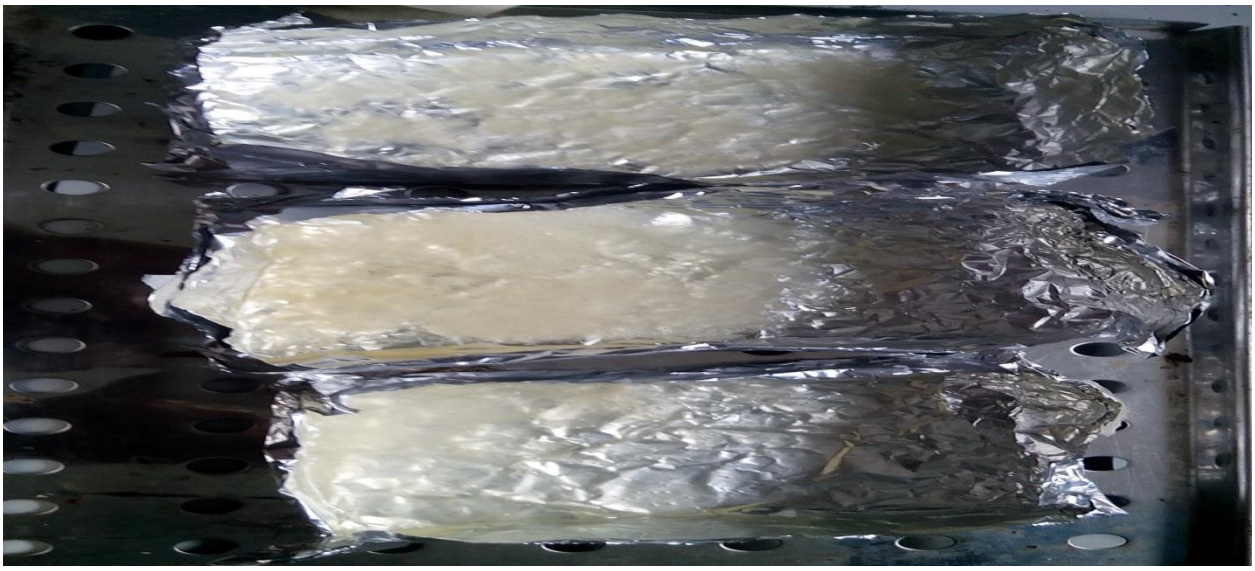

Figure B1-9 The gelatinous pectinous matter before drying

Figure B1-10 On the left dried pectin from Lisbon variety and on the right dried pectin from Mexican variety