

Research Article

Study on the Effect of Extraction Parameters and Precipitation Solvents on Pectin Yield from Lemon Peels

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Abstract

This study was aimed to extract pectin from lemon peel and investigates factors affecting the percentage yield of pectin. Pectin is extensively employed in a variety of food processing procedures as a gelling agent, thickening, emulsifier, and stabilizer. Chemically, it is a polysaccharide, which is a compound that all land plants' cell walls contain in varying amounts. The extraction conditions, such as extraction time, temperature, pH, MLR, and type of extraction solvents, significantly affect the yield of pectin. Pectin was extracted with acid hydrolysis techniques and the extracts were then precipitated with methanol, ethanol and acetone solvents and the effects of these factors on pectin yield were studied. Optimization of extraction parameters has been carried out with OFAT experimental design based on maximum yield. Higher yield of pectin were obtain at extraction temperature of 90°C (36.5%), extraction time of 90 min (38%), material-to-liquor ratio of 1:40 (34.75%) and pH of 2 (31%). Ethanol precipitation has given higher pectin yield than methanol and acetone. The extracted pectin was characterized for its chemical composition, solubility of dry pectin in cold and hot water and alkali, color and thermal properties. It was revealed from this study that dry pectin dissolves in hot water and alkali as well as in cold alkali. The extracted pectin shown moisture content of 13.4%, volatile compounds of 82.3%, ash content of 3.1%, fixed carbon of 14.6% and pH of 3.5 at 30°C were obtained. Hence, it can be concluded that the citrus lemon peel is a potential source of pectin for commercial purpose.

Keywords

Lemon Peel, Pectin, Acid Hydrolysis, Extraction Parameters, Solvents, Yield

1. Introduction

Citrus plants belonging to the family Rutaceae which include fruits such as orange, mandarin, lime, lemon, sour orange and grape fruit appear as a well-known promising source of multiple beneficial nutrients for human beings [1]. Lemon (Citrus Limon from Rutaceae) is one of the citrus fruits, most commonly grown tree fruit in the world. Citrus fruits, which consist of two parts namely the peels (rind skin) and pulp. These two parts are easily separated from each other with the

pulp serving as the edible parts of the fruit while the peels as a good source of pectin [2, 3].

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 [4].

Lemon is a pale yellow, elliptical or globe shaped berry fruit. Citrus fruit, in general contain sugar, polysaccharide,

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Received: 4 August 2025; **Accepted:** 15 August 2025; **Published:** 3 September 2025



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organic-acid, lipids, carotenoids, vitamins, minerals, flavonoids, bitter lemonoids and volatile compounds. Lemon is a good source of potassium, calcium & vitamin C. [5-8]. Citrus by-products, if utilized fully, could be major sources of phenolic compounds. The peels, in particular, are an abundant source of natural flavonoids, and contain higher amount of phenolics compared to the edible portions [7-9].

Lemon (*Citrus sinensis*) peel is an agro-horticultural waste produced in huge quantities from various fruit processing industries. It is normally discarded and dumped in the environment that can create environmental concerns [1, 7-9].

The peel is a by-product of lemon juice processing, with a high potential use. Two different tissues are found in what is colloquially called lemon peel, flavedo and albedo [10]. Flavedo is the peel's outer layer, whose colour varies from green to yellow. It is a rich source of essential oils [11], which have been used since ancient times by the flavour and fragrance industry [12]. Albedo is the major component of lemon peel, and is a spongy and cellulosic layer laid under flavedo. The thickness of the albedo fluctuates according to several variables, among them variety and degree of ripeness [13].

Pectin is a valuable by-product which can be extracted from fruit wastes [14, 15]. Pectin is the methylated ester of polygalacturonic acid that contains 1, 4-linked ∞ -D-galacturonic acid residues [2, 16, 17]. It is commonly found in the cell walls and middle lamellae of higher plants. These polysaccharides consist of 300-100 chains of galacturonic acid units [18, 19].

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 [20]. It is also used in pharmaceutical, dental and cosmetic industries for its jellyfying properties. It is generally produced by acid extraction of citrus peel followed by filtration and precipitation by alcohol [3]. There are three different extraction techniques of pectin from citrus peels [21, 22].

Recently developed extraction methods like Acid extraction method, Direct boiling (hot water) extraction method, Thermo mechanical extraction method, Microwave extraction method has been used for pectin extraction. In direct boiling extraction method a very low yield of pectin obtained from dried lemon peel was reported using distillation apparatus as extracting at 85-90%. In thermo mechanical extraction method obtained yield is poor and require the chemical aids. This method use high pressure boiler so it needs high steam consumption [23, 24]. Microwave heating builds up considerable pressure inside a material and gave higher rate, amount of extraction [25] In acid extraction method commonly used

acidifying materials are mineral acids including, hydrochloric, sulphuric and phosphoric acids. We have selected the acid extraction method for pectin extraction because it is new technology, simple to construct and operate at minimum cost [26].

The yield of pectin usually depends on the extraction conditions, such as temperature, extraction time, pH, type of extraction solvents [18], and drying [27].

The scope of the present work was to extract pectin from discarded lemon peel by acid hydrolysis (HCl) and precipitation using solvents. Factors affecting the percentage yield of pectin were studied. Characterization of the extracted pectin to determine the solubility of dry pectin in cold and hot water, the colour of the pectin, ash content, moisture content and percentage yield of the pectin.

The aim of this paper is to investigate the effect of solvent types and extraction conditions on pectin yield extracted lemon peels and to determine optimum conditions for maximum extraction of pectin, and to study the physico-chemical properties of the pectin extracted from the lemon peels.

2. Materials and Methods

2.1. Materials and Equipment's

The materials used to conduct this study include fresh citrus peels of lemon.

The apparatus used in this study such as electronic balance, beakers, measuring cylinder, stove, magnetic stirrer, thermometer and digital pH meter. The equipment used in this study were Whatman paper, Electric Blender, Sieve analyzer, Oven dry, TGA 4000 Perkin Elmer etc.

2.2. Chemicals

All the chemicals and solvents used in this study are laboratory grade reagents. Hydrochloric acid was used for the extraction. Ethanol, Methanol and Acetone were used for precipitation of pectin during extraction. Distilled water, sodium hydroxide etc were also used.

2.3. Preparation of Lemon Peels

Lemon was collected from the nearby areas of kombolcha city and the collected lemons were washed thoroughly with tap water to remove impurities and dirt on the surface. Then the peels were separated from the lemon and again the peel was washed to remove the remaining impurities. The lemon peels were then dried by exposing to sunlight and the dried lemon peel was subjected to fine powder by grinding. The grinded lemon peel was again dried at shade.



Figure 1. Sample material preparations.

2.4. Determination of Particle Size

Particle size of the grounded lemon peel powder was determined using sieve analyzer. In this study a mechanical sieve shaker was utilized. The grounded lemon peel powder were first dried and then subjected to sieve analysis. Dry sieving was used to know and decide the particle size of the material and was done by shaking or vibrating. The mechanically dry ground particle size of lemon peel powder was investigated by a sieve analyzer. The fine powder was produced at 60 micrometers (μm) in size.

2.5. Pectin Extraction

The pectin from the plants bi-products (lemon peel) were extracted by using acid extraction techniques. Hydrochloric acid was used for the extraction of pectin. The extracted pectin was precipitated with solvent. Different types of solvent such as ethanol, methanol and acetone were used for precipitation of pectin. Then the best type solvent and optimization conditions were identified based on extraction efficiency and was proposed in this investigation.

Optimization of extraction conditions like extraction temperature, extraction time, MLR and pH were carried out based on maximum extraction efficiency of the pectin. OFAT experimental design was applied for optimization of the extraction conditions. Extraction were tried at 30, 60 and 95 °C of extraction temperature; at 40, 60, and 90 minutes of extraction time; at 10, 25, and 40 of material to liquor ratio; at 2, 3.5 and 5 of pH level using hydrochloric acid as extracting agents and effect of this factors on pectin yield were studied. The extracts

were then precipitated with methanol, ethanol and acetone solvents and effect of these factors on pectin yield was studied. The variables were optimized using OFAT experimental design.

2.5.1. Percentage Yield of Pectin

Percentage yield of pectin was determined using equation (1) given below:

$$Y_{\text{pec}} (\%) = \frac{P}{B_i} \times 100 \quad (1)$$

Where, $Y_{\text{pec}} (\%)$ is the extracted pectin yield in percent (%), P is the amount of extracted pectin in grams and B_i is the initial amount of lemon peel.

2.5.2. Methods of Extraction

The sequence of the operations performed for the extraction of pectin from lemon peel was presented in Figure 2.

Method for extraction of pectin consisted of adding 300 ml of distilled water to 10 g lemon peel powder (LPP) in a beaker and addition of hydrochloric acid adjusted at different pH (2, 3.5, and 5) and then heating the mixture to 40 °C, 60 °C and 95 °C for this different treatment time 30, 60 and 90 minutes, in a hot water bath. This means that separately different extractions have been carried out in order to optimize the extraction parameters. Thereafter, the peels were removed from the extracts by filtering through a filter paper/muslin cloth. The pectin extract obtained from different extractions, after cooling to room temperature, was precipitated with 95% ethanol/methanol/acetone and then with thorough stirring, left for 1 hrmin to allow the pectin float on the surface. The ge-

latinous pectin flocculants was then skimmed off. The extracted pectin was purified by washing in 20 mL ethanol/methanol/acetone and then 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. Finally, after drying the pectin was ground. The resulting pectin was weighed using a digital weighing electronic balance. Finally percentage yield of pectin was calculated according to the given formula above (Equation (1)).

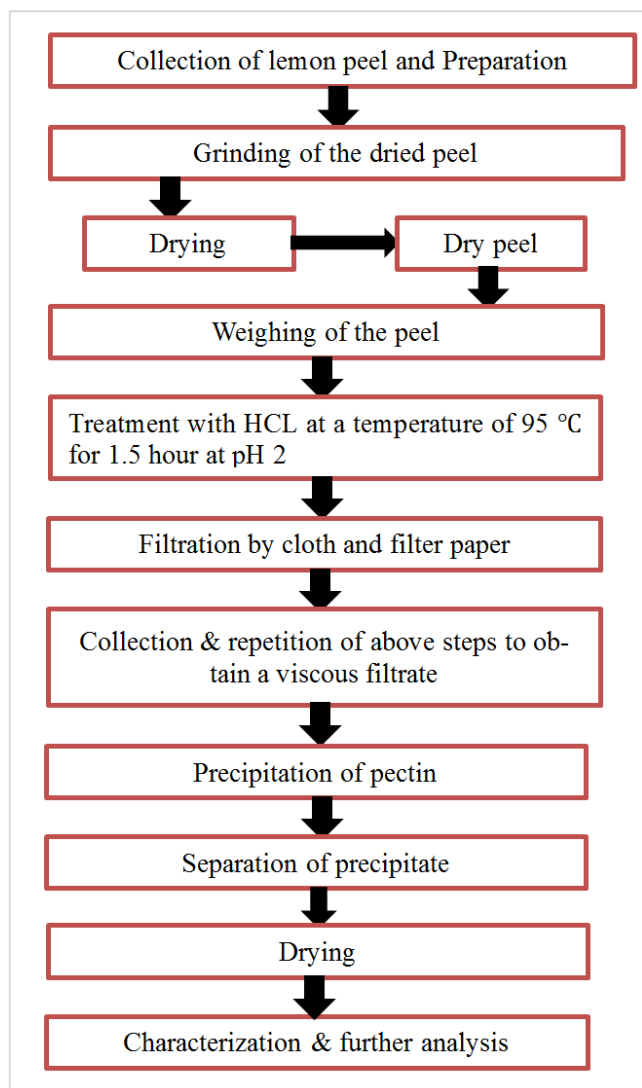


Figure 2. Sequence of operations for the extraction of pectin from lemon peel.

2.5.3. Isolation of Pectin

Isolation of pectin was carried out using ethanol or methanol or acetone as precipitating agent. Ethanol or methanol or acetone was used as a precipitating agent for pectin. For this purpose, twice amount of ethanol/methanol/acetone was added to the cooled solution and continuous stirring was done for 20 min. Then the mixture was kept aside for 1 hr without

stirring. Pectin was filtered through four layered muslin cloth. The precipitate was washed 2 to 3 times by ethanol/methanol/acetone, to further remove any remaining impurity. Finally, precipitate was kept for drying at 40 °C to 50 °C in hot air oven, and percentage yield was determined. It was then stored in desiccators until further use.

2.6. Characterization

The extracted pectin was characterized for its chemical composition, color, solubility and thermal properties. The percentage yield of pectin obtained from lemon peels were determined and proposed.

2.7. Qualitative Test

2.7.1. Pectin Colour

Visual inspection of dried pectin samples was conducted, and the colors of the samples were recorded.

2.7.2. Solubility in Hot and Cold Water (Dry Pectin)

The first step was to place 0.05 g of the pectin samples in various conical flasks, then add 10 ml of 95% ethanol and 50 ml of distilled water. The resulting mixture was violently shaken to create a suspension, which was then heated using a magnetic stirrer for 15-20 minutes at 90-95 °C.

2.7.3. Solubility in Hot and Cold Alkali (NaOH)

Initially, 0.1 g of dry pectin was added to 10 ml of 0.1 N NaOH and the mixture was heated at 90-95 °C for 15 minutes using a magnetic stirrer.

2.8. Chemical Composition

2.8.1. Determination of Moisture Contents

The moisture contents of the pectin samples were determined using the ASTM D6304 procedure. 5 gm of the sample was weighed and dried by employing an electric oven at 105 °C for 12 hr to a constant weight. The weight loss was taken and calculated as a percentage of moisture (%). The percentage of moisture content were determined as given in equation (2):

$$\text{Moisture Content (\%)} = \frac{(\text{Weight of original of pectin} - \text{Weight of dry pectin}) \times 100}{\text{Weight of dry pectin}} \quad (2)$$

2.8.2. Determination of Ash Contents

The ash content was measured by igniting sample in a muffle furnace. 2 g of the sample was weighed and burned in a furnace at 600 °C for 5 h and the remaining was weighed, and the percentage of ash content (%) was calculated. Ash content was calculated as per equation (3):

$$\text{Ash content (\%)} = \frac{(W_1 - W_2)}{W_n} \times 100 \quad (3)$$

Where, W_1 (gm) - weight of moisture-free sample before ignition; W_2 (gm) - weight of sample after ignition; W_n (gm) - oven dry weight of sample, gm.

2.8.3. Volatile Matter

The volatile matter was determined using muffle furnace. To measure the volatile content, known content of dried sample (10 gm) was taken in a closed crucible and kept inside the muffle furnace at 660 °C for 5 min. The loss in weight of

the sample was found out and the percentage of volatile matter was calculated as per equation (4):

$$\text{Volatile Matter (\%)} = \frac{\text{Loss in weight of the sample (gm)}}{\text{Weight of moisture-free sample (gm)}} \times 100 \quad (4)$$

2.8.4. Fixed Carbon

The fixed carbon of samples was calculated by subtracting the sum of ash content (%) and volatile matter (%) from 100. The fixed carbon is the residue left after removing the volatile matter and the ash from the substance.

$$\text{Fixed carbon (\%)} = 100 - (\text{volatile matter (\%)} + \text{ash content (\%)}) \quad (5)$$

2.9. Thermal Analysis

The thermal behaviour of the extracted pectin were analysed using Thermogravimetric Analysis (TGA 4000 Perkin Elmer). Sample weighing 10 mg was analysed under a nitrogen atmosphere with 20 ml/min of gas flow rate, heating rate of 10°C/min, and a temperature range from 25°C to 600°C. TGA analysis was used for thermal analysis of the prepared pectin based binder as a means to decide the curing temperature.

3. Result and Discussion

In this study, the effect of extraction temperature, extraction time, MLR and pH condition on the percentage yield of pectin from lemon peel were investigated. The effects of the types of solvents used for precipitation of the pectin on the percentage yield of pectin were also studied.

Extraction of pectin is influenced by several variables such as extraction time, pH, temperature, solid/solvent ratio. Beside this the alcohol type used for precipitation of pectin during extraction process affect the pectin yield.

3.1. Effect of Extraction Temperature on Pectin Yield

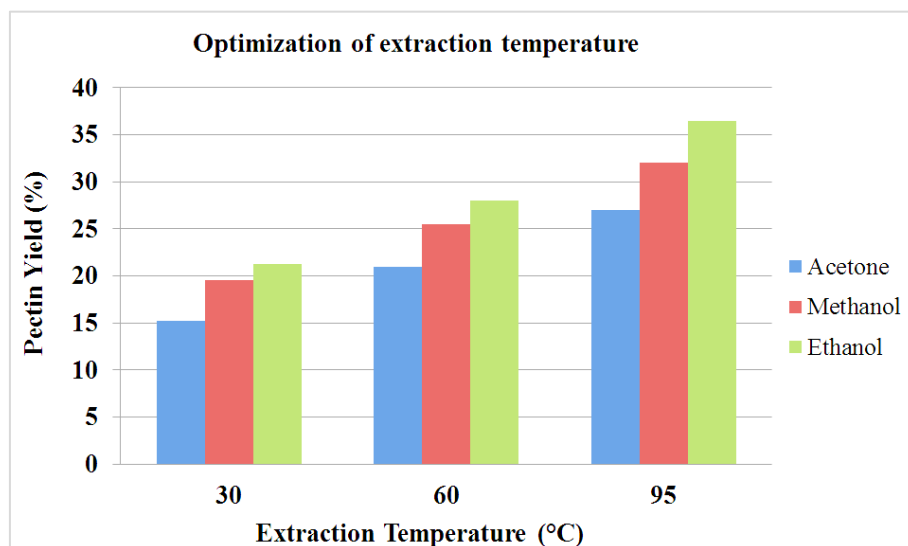


Figure 3. Effect of extraction temperature on pectin yield.

Extractions of pectin from lemon peel were carried out at 30°C, 60°C and 95 °C by keeping other factors constant and the pectin yield was given in Figure 3. The acid hydrolyzed lemon peel powders were precipitated with different types of

solvents like acetone, methanol and ethanol to obtain pectin.

It was observed from Figure 3 that as extraction temperature increases the pectin yield increased. It indicated that temperature has a significant effect on pectin yield percentage.

Ethanol precipitated pectin given higher pectin yield as compared to acetone and methanol. In all extraction temperatures the amount of pectin obtained was higher with the ethanol precipitated pectin. It was revealed that the percentage yield of pectin at 95 °C extraction temperature resulted higher pectin yield.

3.2. Effect of Extraction Time on Pectin Yield

To optimize the extraction time, extraction of pectin were investigated at 40, 60 and 90 min by keeping other parameters constant and the pectin yield was given in Figure 4. The effect of solvent types used for the precipitation of the pectin was also studied.

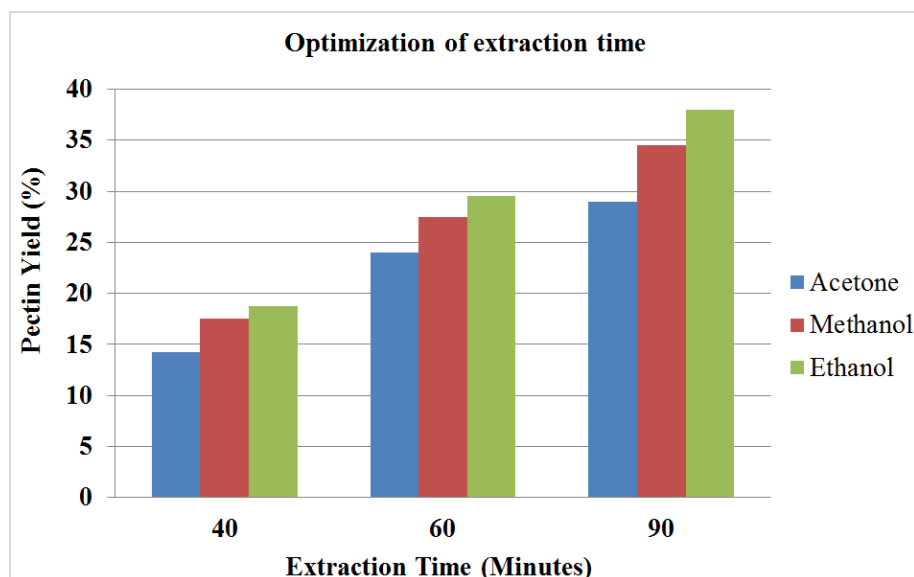


Figure 4. Effect of extraction time on pectin yield.

From Figure 4 it was shown that as extraction time increases, the percentage yield of pectin increased. The higher pectin yield was obtained at 90 minutes extraction time. Ethanol precipitated shown higher pectin yield as compared to

acetone and methanol. Lemon peel powder treated at 90 minutes and precipitated with ethanol given higher pectin yield. Time has a significant role in the percentage yield of pectin.

3.3. Effect of Extraction MLR on Pectin Yield

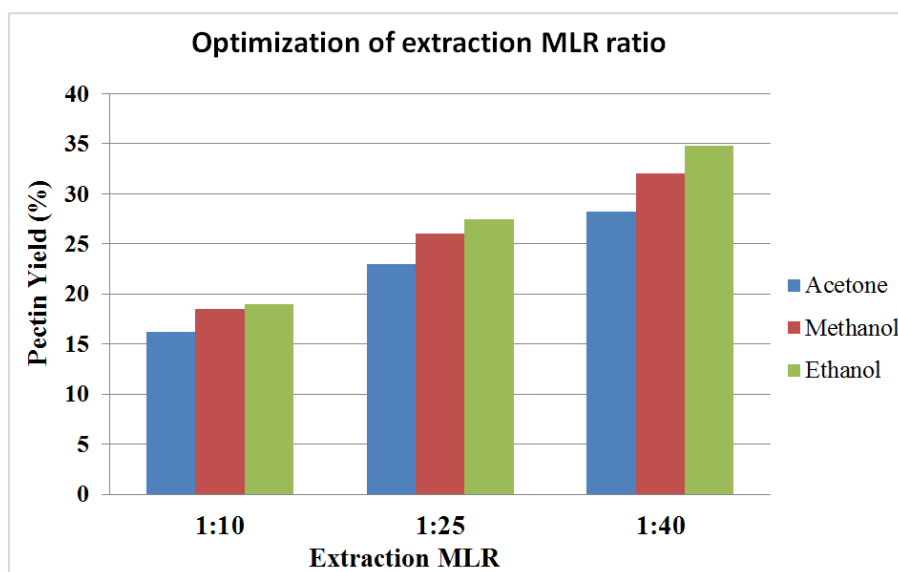


Figure 5. Effect of extraction MLR on pectin yield.

Optimization of extraction material to volume ratio were tried at 1:10, 1:25 and 1:40 by keeping other parameters constant and the effect of MLR on pectin yield were investigated. Different solvent types were used for the precipitation of pectin and their effects on percentage yield were also studied. The percentage pectin yield was given in Figure 5.

The above Figure 5 indicates that MLR has a significant effect on the percentage yield of pectin. Higher percentage pectin yield was obtained at 1:40 MLR. Ethanol precipitated given higher pectin as compared to acetone and methanol. In all extraction MLR the ethanol solvent precipitated pectin shown better pectin yield as compared to other solvents.

3.4. Effect of Extraction 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, hydrochloric acids were used in this study.

The extractions of pectin were carried out with acid extraction techniques. In this study the extraction pH conditions were tried at 2, 3.5 and 5 pH levels by keeping other factors constant.

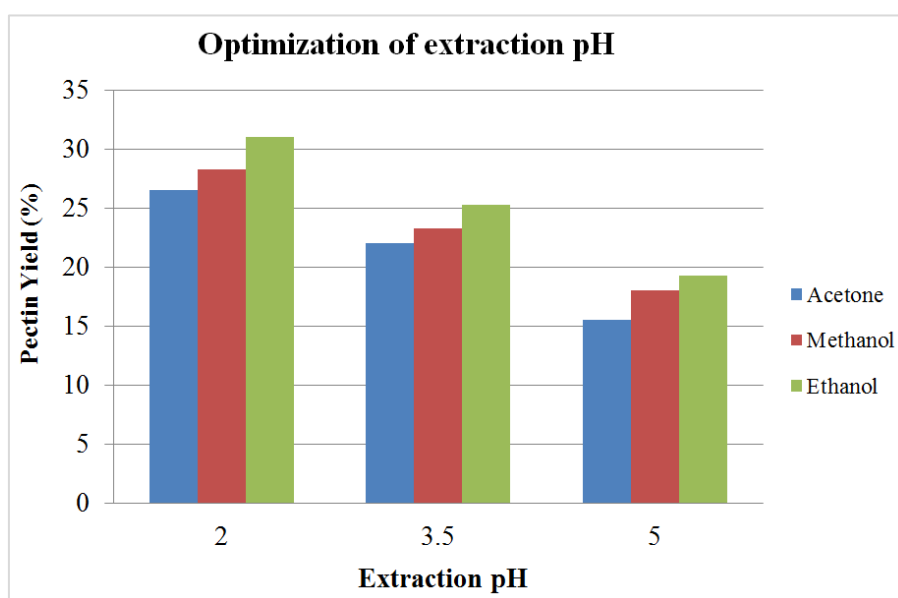


Figure 6. Effect of extraction pH on pectin yield.

From the above Figure 6 it was observed that as acid pH strength increases the percentage yield of pectin increases. At strong acid pH, higher pectin yield were obtained. In all extraction pH conditions the ethanol precipitated given higher pectin. At pH of 2 (strong acidity), higher pectin yield was obtained.

Figure 6, it is evident that pectin extraction at pH 2 gave significantly higher yield than that of pH 3.5, and 5. This is likely due to the enhanced ability of acid in solubilizing the proto pectin from the albedo with an increase of acid strength. Acids are generally the strongest extracting agents with regards to the yield of extracted pectin.

3.5. Pectin Colour

Qualitative test were performed to identify the color of the pectin. The dried pectin samples were observed visually and the colour of pectin shows white and shown in Figure 7. However, some of the acetone precipitated pectin shows

yellowish.



Figure 7. The Extracted wet pectin.

3.6. Qualitative Test Analysis

Solubility of dry pectin in cold and hot water as well as solubility of dry pectin in cold and hot alkali were assessed and given in Table 1.

Table 1. Solubility in cold and hot water and alkali.

S. No.	Parameters	Solubility
1	Solubility in cold water	Insoluble and forms suspension after vigorous shaking
2	Solubility at 90- 95 °C for 15-20 min	Dissolve
3	Solubility of pectin suspension in cold alkali	Forms a precipitate
4	Solubility of pectin suspension in hot alkali	Dissolve

The pectin obtained from lemon peel powder was found soluble in hot water, and soluble in cold and hot alkali. However dry pectin is insoluble in cold water but forms suspension after vigorous shaking.

3.7. Chemical Analysis

Chemical composition such as moisture content, ash content, volatile matter, fixed carbon and pH of the extracted pectin were evaluated. The determined chemical compositions of the pectin extract were presented in Table 2.

Table 2. Chemical composition of pectin.

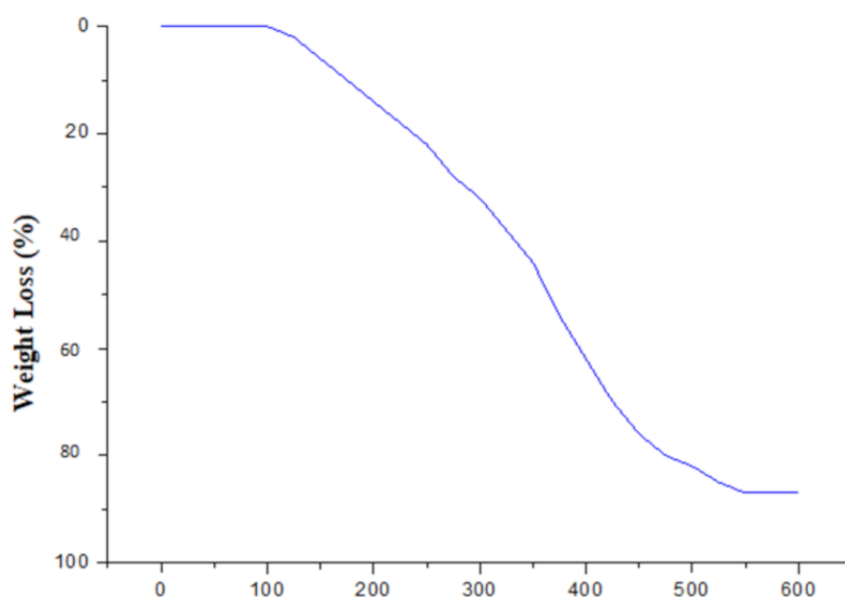
Parameters	Observed Value
Moisture content	13.4%
Total ash	3.1%
Volatile matter	82.3%

Parameters	Observed Value
Fixed carbon	14.6%
pH	3.5

The result of Table 2 showed that the pectin had a moisture content of 13.4%, volatile compounds of 82.3%, total ash content of 3.1%, fixed carbon of 14.6% and pH of 3.5 at 30 °C. The result obtained showed that the amount of ash content of pectin was small. The material has high volatile compound and moderately fixed carbon content which may be due to the high amount of hydrocarbons in the pectin.

3.8. Thermal Analysis

The thermal properties of the prepared pectin based binder were assessed in Thermogravimetric analysis and shown in Figure 8. Thermal characterization of the prepared pectin by using TGA is required in order to understanding the nature of thermal dissociation of the pectin.

**Figure 8.** Thermal analysis of extracted pectin by TGA.

The TGA curves in Figure 8 showed an initial small mass loss of around 100 °C, which could be attributed to the evaporation of absorbed moisture from the pores of extracted pectin. Another major weight loss was observed between 172 °C due to evaporation of volatile matter. Between 232 °C - 425 °C there is weight loss observed which was due to macromolecule degradation of extracted components. Lastly, above a temperature 536 °C there is degradation and converted to ashes.

From Figure 8 it was observed that the pectin thermal stability increased gradually with increasing temperature, which could be explained by the existence of various active groups like carboxylic and hydroxyl groups, which generate new crosslinks that prevent degradation at higher temperatures.

4. Conclusion

In this study extractions of the pectin were carried out with acid hydrolysis methods from lemon peel and factors affecting percentage yield of pectin was studied. In this extraction techniques optimization of the extraction conditions were carried out with OFAT experimental design. It was observed that, the optimized extraction conditions were 95 °C extraction temperature, 90 minutes extraction time, 1:40 MLR and pH 2 with hydrochloric acid. At this optimized extraction conditions higher percentage yield of pectin was obtained. It was also revealed that ethanol precipitation given higher pectin yield as compared to other solvents. From this investigation it was concluded that temperature, time, MLR, PH and solvent type used has significant effect on percentage yield of pectin. Pectin extraction on a dry basis, as opposed to a wet basis, is advised for higher yields. Chemical composition of pectin were determined and a moisture content of 13.4%, volatile compounds of 82.3%, total ash content of 3.1%, fixed carbon of 14.6% and pH of 3.5 at 30 °C were obtained. From this investigation it was observed that extraction parameters and precipitation solvent types has significant effect on yield of pectin.

Abbreviations

ASTM	American Society for Testing and Materials
TGA	Thermogravimetric Analysis
OFAT	One-factor-at-a-time
MLR	Material to Liquor Ratio
LPP	Lemon Peel Powder
HCL	Hydrochloric Acid

Acknowledgments

The authors wish to thankful Kombolcha Institute of Technology (KIOT), Wollo University and Bahir Dar University for providing the laboratory facilities.

Author Contributions

Asaye Dessie: Conceptualization, Investigation, Writing – original draft, Writing – review & editing

Lami Amanuel: Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Writing – review & editing

Ashenafi Alebachew: Conceptualization, Formal Analysis, Resources, Writing – review & editing

Funding

The research, authorship, and/or publication of this paper were all done without any financial assistance from the author(s).

Data Availability Statement

The corresponding author can provide the data that were utilized to support the study's conclusions upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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