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ORIGINAL ARTICLE

Extraction And Characterization of Pectin From Lemon Peels Waste

Javeed Akhtar[1], Mebrhit Gebremariam Abrha[1],P.K. Omre[2],Gebremeskel Gebrekirstos Gebru[1]
[1]Department of Chemical Engineering, College of Engineering and Technology,Adigrat University, Adigrat, Ethiopia.
[2]Professor & Head, Department of Post Harvest Process and Food Engineering, College of Technology, G.B.P.U.A.&T, Pantnagar, Uddhamsingh Nagar, Uttarakhand , India
Email ID er.jakhtar@gmail.com

ABSTRACT
The aim of this study was to extract pectin from peel waste of variety of citrus fruit namely lemon (Citrus lemon) and to investigate the effect of processing conditions on the process of extraction. The raw material was prepared and pectin was extracted using nitric acid at three different temperatures (40 °C, 60 °C and 80 °C), and PH (1.0, 2.5 and 4.0). The experiments were carried out in water bath for two hours. Design-Expert at three-level three -factor general factorial design was applied. A total of 27 experiments were conducted for the 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 the variety varies from 4.69% -20.36%. 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 20.36% was obtained at PH 1.0 and temperature of 60 °C for the variety. The best condition for extraction using nitric acid was at 60 °C for 2 hours at pH 1.0. The pectin obtained from these methods was compared in terms of yield, physicochemical properties. 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 and varied from 3.08-3.39%, 8.59-8.62%, 312.5-384.6%, 3.44-4.3%, 65.31-80.76%, 29.973-30.23% for variety respectively.
Key words: *Pectin; Citrus peel; Processing conditions: Extraction; Yield*

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INTRODUCTION

Lemons are scientifically known as Citrus lemon. Lemon is the third most important species of citrus after orange and mandarin, with a high production. Argentina is currently the world's largest producer of lemons [8]. The peel is a by-product of lemon juice processing, with a high potential use. Lemon contains different layers namely flavedo and albedo. Flavedo is the peel's outer layer, whose color varies from green to yellow. It is rich in essential oils [4], which have been used since early times by the flavor and fragrance industry [24] whereas albedo is the main constituent of lemon peel, and is a spongy and cellulosic layer laid under flavedo. In Additionally, the presence of flavonoids and vitamin C with antioxidant properties in fresh lemon albedo includes healthier benefits than other dietary fiber sources [13].

Citrus peels are the most important sources of pectin, which is also rich in agro industrial subproducts such as sugar beet pulp, pulps of grapes, peach peels and pumpkin. Fewer commonly, pectin can also be extracted from by-products of the manufacture of starch from potatoes, sunflower heads in oil making and onions [18].

Pectin is commercially extracted from pectin-rich sugar-beet pulp, apple pomace and citrus peels because it is an anionic plant cell wall polysaccharide based on a-(1–4) linked D-galacturonic acid [22].

Pectin's a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants and it can be extracted from lemons peels. Much smaller proportions of these substances are found in the cell walls of grasses. The maximum pectin concentrations are originated in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the

plasma membrane. Pectin is a refined carbohydrate product achieved from the internal portion of the rind/peels of citrus fruits. It consists chiefly of partially methoxylatedpolygalacturonic acid [9].

One of the major problems challenging the food industry throughout the world is how to make full utilization of the agriculture waste material. Among the many agricultural wastes there is lemon fruit waste that contains many useful components like pectin which can be extracted and utilized in different products. Hence, lemon peel has become one of the most important sources of commercial pectin [10].

Pectin is capable of forming gels with sugar and acid under suitable conditions. It is formed almost universally in plant cell of all species suitable for use in the production of sugar jellies and industrial production of apple pomace, citrus peels and sugar beet chips. Although pectin occurs commonly in most of the plant tissues as a cementing substance in the middle lamella and as a thickening on the cell wall, the number of sources that may be used for the commercial manufacture of pectin's is very limited. Pectin is capable of forming gels with sugar and acid the structure of pectin is very difficult to determine because pectin can change during isolation from plants, storage, and processing of plant material.

In present study, lemon peel was used as raw material and pectin was extracted from this raw material using water based extraction technique. Effective solid waste management is one of the most essential elements for an industry to achieve a sustainable development. Improper treatment of waste will affect peoples' health and the environment. For the citrus processing industry the disposal of fresh peels has become a major concern for many years lemon peels are the major solid by-product of the overall process. Lemon peels are the major source of lemon peel oil and these oils can be used for various industrial application. In order to produce good quality of extracts or essential oil from the lemon peel waste, the most appropriate extraction technology must be applied.

MATERIAL AND METHODS

Chemicals and equipment

Chemicals and reagents used:

The following analytical grade chemicals were used for extraction of pectin. These include nitric acid, phenol red indicator, hydrochloric acid (HCl), sodium hydroxide (NaOH), distilled water, sodium chloride (NaCl) and ethanol. All the chemicals and reagents were provided by Addis pharmaceutical factory which is found in Adigrat, Tigray, Ethiopia.

1. Distilled water: used for extraction of pectin
2. Ethanol: Used for precipitating agent.
3. Powdered peel: the major raw material of pectin extraction.
4. Hydrochloric acid: used for determination of Methoxyl content
5. Phenol red: used for determination of equivalent weight
6. Nitric acid: used for adjusting PH value.
7. Sodium hydro oxide: used for determination of methoxyl content

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. The equipment are described in table 1.

Sample preparation

Mature lemon fruits were purchased from the local market of Adigratcity, Ethiopia. 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 manually. After juice extraction the peel was chopped into small pieces in a vegetable cutter dried at 60 °C for 48 hrs in an oven followed by grinding into powder using centrifugal mill. The powder was then sieved using sieve.

Table 1. Equipment description

$S.N_O$	Equipment	Use
1	Mortar and pestle	For grinding the peels
2	Sieve	For meshing
3	Balance	To measure sample
4	Water bath	Used for heating purpose
5	PH meter	Used to measure pH value
6	Cheese cloth	Used for filtration or separate cake and filtrate
7	Knife	used for cutting to reduce the peel
8	Beaker	Used us storage of mixture and For measuring liquid solution
9	Oven	For drying sample to get pectin
10	Furnace	For burning to get ash content
11	Filter paper	Used for filtration
12	Stirrer	For perfect mixing
13	Aluminum foil	Used to cover mouth of beaker
14	Centrifugal mill	For grinding in to powder
15	Measuring cylinder	To measure volume
16	Round bottomed flask	Used as laboratory glassware
17	Funnel	For guiding liquid
18	Micropipette	To deliver or measure volumes of liquid
19	Beakers	For mixing or heating
20	Centrifuge	To separate solid from liquid

Method

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(HNO_3) adjusted at different PH(1,2.5 and 4) and then heating the mixture to (40, 60 and 80)°C for (1, 2 and 3) hr 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 hr 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 hr. After drying the pectin was ground. The dried pectin obtained from the lemon peel was packed in food grade polythene bags and stored in a cool dry place until further analysis. Finally percentage yield was calculated [2].

Process flow description

Process flow description is given below and figure 1 shows the process of pectin extraction:

Collection of raw material: The raw materials such as lemon peels were collected from local market.

Washing with water: The collected lemon peel was washed with water to remove impurity. Cutting the peel: the peel was cut to dry with short period of time

Drying: removing moisture as much as possible for grinding

Weighing the peel: to know how much water is removed and also how much sample used for the extraction

Grinding: making piece size to extract more using mortar and pestle.

Mixing: making solution by perfect mixing

Heating: This process was done in water bath at temperature of 40, 60 and 80^{o}C for 1, 2 and 3 hours at PH 1.0, 2.5, and 4.0 to heat the solution for further extraction.

Filtration: separation of filtrate from cake by using vacuum filter.

Separation of precipitate: after the filtrate was settled down the precipitate was removed.

Drying: in this process the final filtrate was dried at temperature of 40, 60 and 80^{o}C for 1, 2 and 3 hours in oven.

Pectin: the final result of the process.

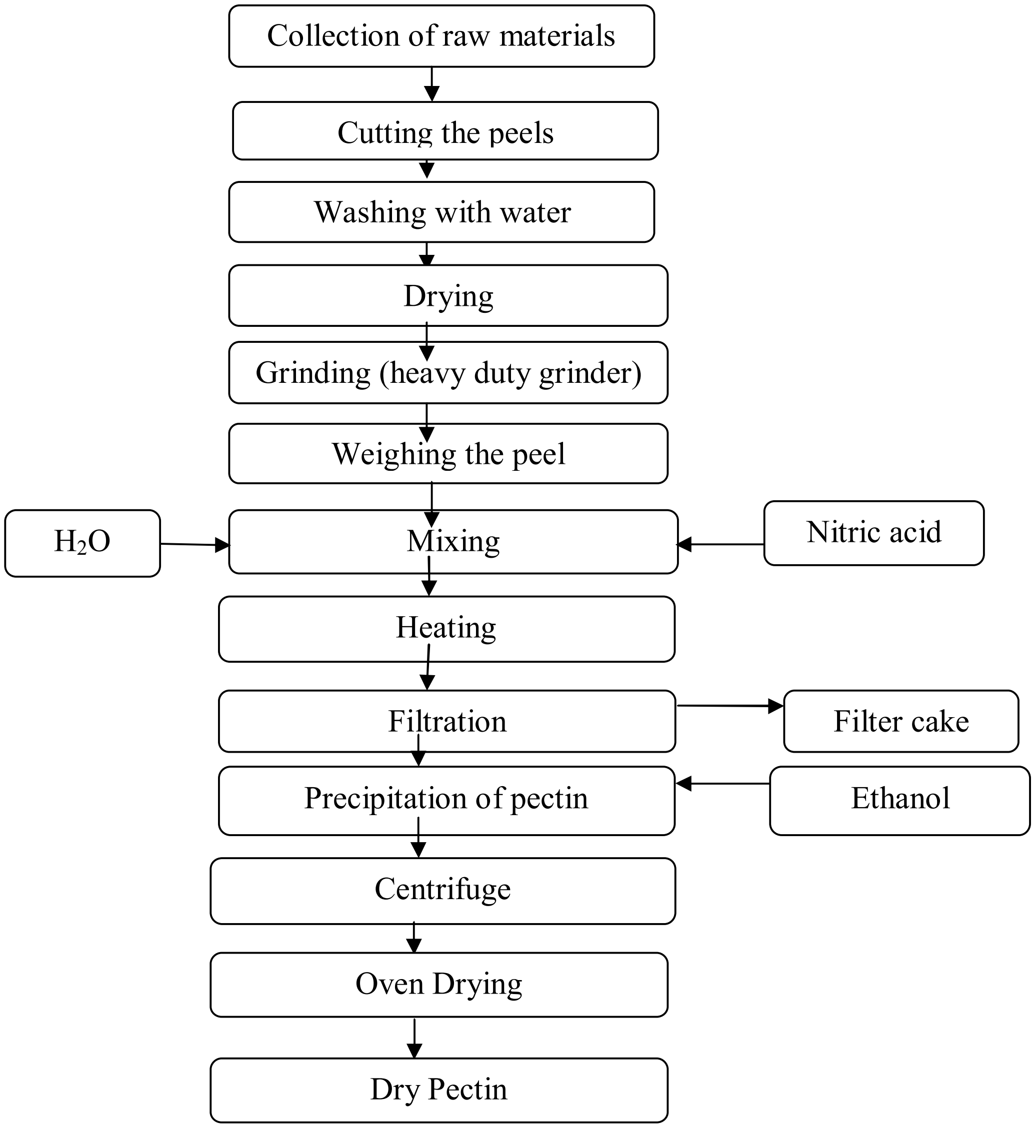


FIGURE 1.process flow diagram of pectin extraction

Experimental design

In present investigation, the total twenty seven experiments were conducted at different three level of PH (1.0, 2.5 and 4.0), different three level of temperatures(40, 60 and 80) °C and three different level of extraction time of 1, 2 and 3hours for the production of pectin from lemon peels using water based extraction as shown in the below Table. The effect of process parameter such as pH, temperature and extraction time on the different quality characteristics such as moisture content, ash content , Methoxyl Content, equivalent weight, AUA, and degree of esterification. The optimization of the process parameters for pectin extraction was carry out based quality parameters using software tools. The quality of the pectin was analyzed after complete extraction process.

Analysis of quality parameters

Pectin yield percentage

The yield of pectin was estimated using following equation (1).

$$Y_{PEC} = \frac{P}{Bi} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where,

Y_{pec} is the extracted pectin yield in percent (%),

P is the amount of extracted pectin in g and

B_i is the initial amount of lemon peel (5g).

Moisture content

Moisture of the pectin was determined according to AOAC [3]. A clean dried dish was weighed, and 4g 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 reweighed. Then, the moisture content was estimated by the formula:-

Moisture content % $=\frac{W2-W3}{W2-W1}$ *100……(2)

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)

Ash content

Ash content of pectin was determined by Ranganna's method [20]. Weighed 5 g of pectin substance (sample).The sample was ignited slowly, and then heat for 3-4 hr at 500 ºC. Then cooled the crucible to room temperature in desiccators and weighted properly. The process will be weighted till constant weight come and final weight will be noticed.

% ash= $\frac{W2-W1}{W} \times 100$…….. (3)

Where,

W_2 - Final weight of dish and ash,

W_1- Weight of dish,

W- Weight of pectin sample

Equivalent Weight

Equivalent weight is used for calculating the anhydrouronic acid content and degree of esterification. It is determined by titration with sodium hydroxide to pH 7.5 using either phenol red or Hinton's red indicator. Equivalent weight was determined by Ranganna's method [20]. 0.5 g sample was taken in a 250 ml conical flask and 5 ml ethanol was added. 1 g of sodium chloride to sharpen the end point and 100 ml of distilled water were added. Finally 6 drops of phenol red or Hinton's indicator was added and titrated against 0.1 N NaOH. Titration point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content.

Equivalent weight $= \frac{\text{weight of sample}}{\text{ml of alkali} \times \text{normality of alkality}}$…… (4)

Methoxyl Content (MeO)

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

Determination of MeO was done by using the Ranganna's method [20]. The neutral solution was collected from determination of equivalent weight, and 25 ml of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 ml of 0.25N hydrochloric acid was added and titrated against 0.1N NaOH to the same end point as before like in equivalent weight titration.

Methoxyl content % = $\frac{\text{ml of alkali} * \text{Normality of alkali} * 3.1}{\text{weight of sample(g)}}$ …….. (5)

Total Anhydrouronic Acid Content (AUA)

Estimation of anhydrouronic acid content is essential to determine the purity and degree of esterification, and to evaluate the physical properties. Pectin, which is a partly esterified polygalacturonide, contains 10% or more of organic material composed of arabinose, galactose and perhaps sugars. Making use of the equivalent weight and methoxyl content value of titre used. Total AUA of pectin was obtained by the following formula [15].

% of AUA= $\frac{176 \times 0.1Z \times 100}{W \times 100} + \frac{176 \times 0.1Y \times 100}{W \times 100}$…… (6)

Where 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

Determination of Degree of Esterification (DE)

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

%DE= $\frac{176 \times \%MeO}{31 \times \%AUA} \times 100$……(7)

Where;

% MeO = Methoxyl content,

% AUA=Anhydrouronic Acid Content

Laboratory experimental works

FIGURE 2.(A) Lemon collected from local Adigrat city (B) Dried lemon peels

FIGURE3.(A) Meshing of Lemon peels powder (B) pH adjustments

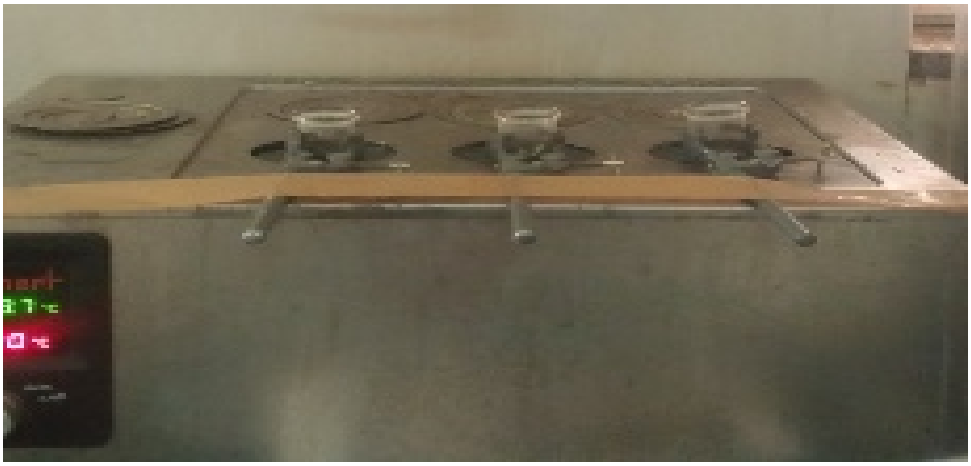


FIGURE4. The solution at water bath

FIGURE5. After addition of 96% ethanol on the clear solution and coagulated pectin

FIGURE 6.Filtration of pectin

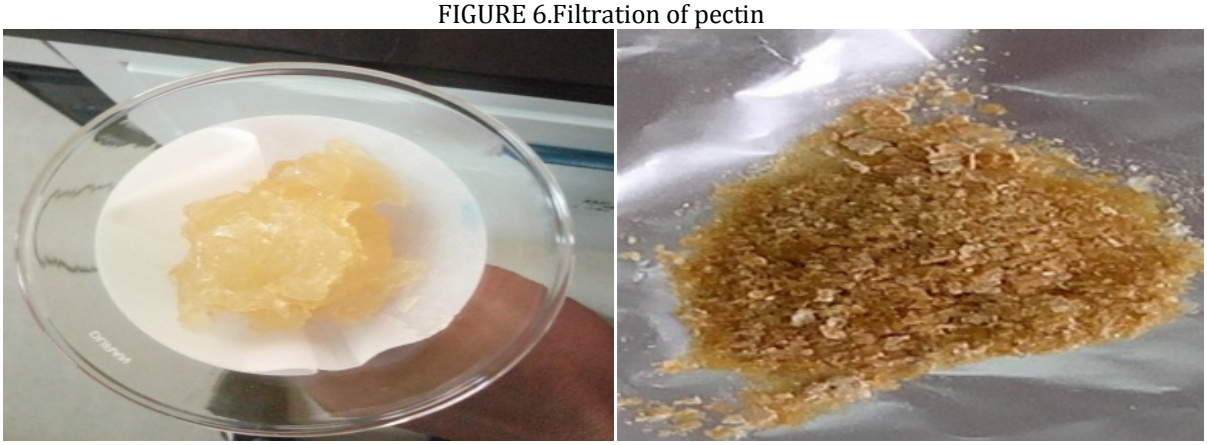


FIGURE7.(A) Pectin before drying (B) Dried pectin

Statistical Analysis
For statistical analysis, the Design Expert 7.0.0 software tool was used to analyze the experimental data.

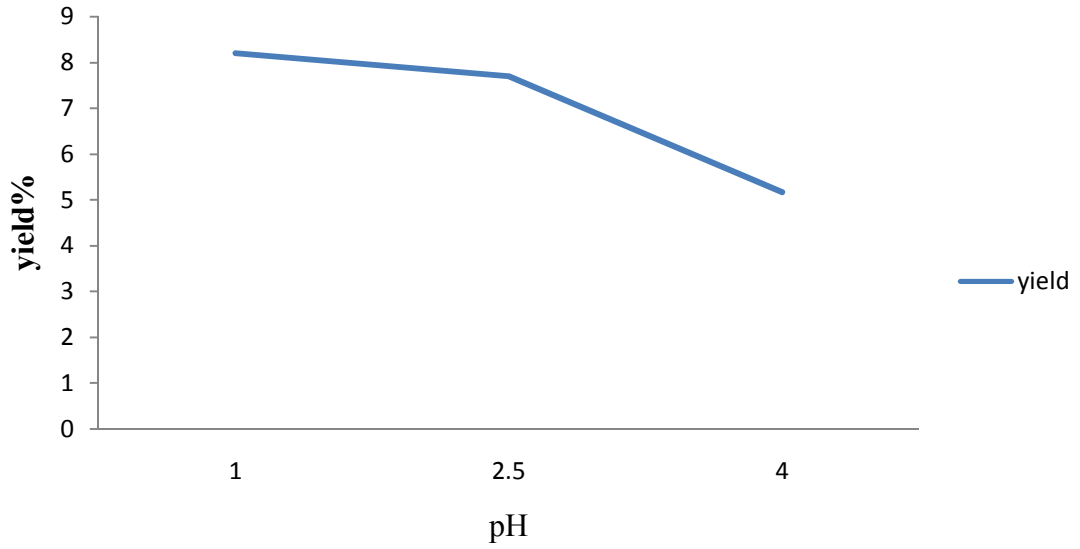
RESULTS AND DISCUSSION
Effect of individual process parameters
It was found that temperature, time and pH significantly affect the percentage yield of pectin.

Effect of pH on pectin yield

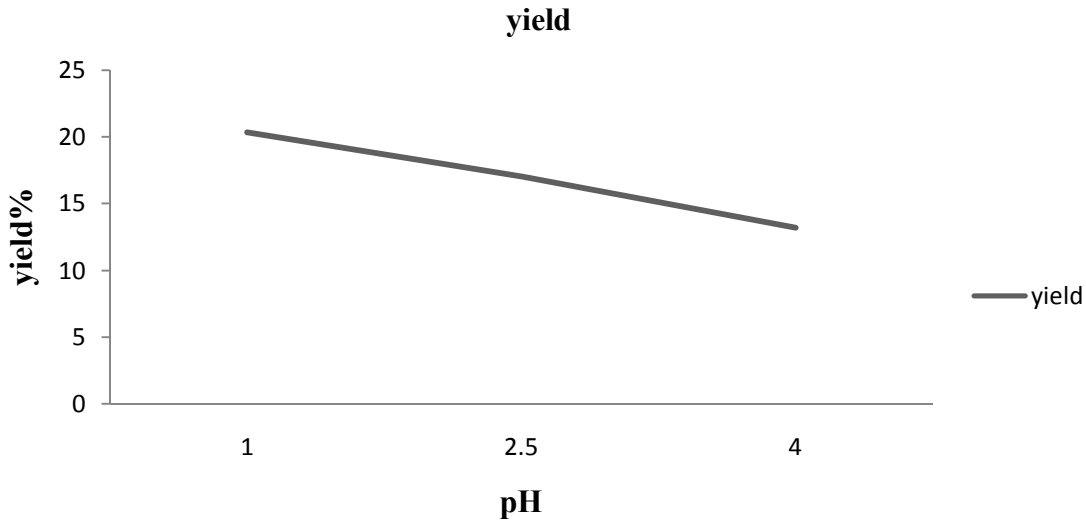
With regards to the extraction parameters, pH is considered as one of the most crucial parameters affecting both the amount and properties of pectin being extracted. It has previously been reported that several acids can be utilized for the extraction of pectin. However, mineral acids such as sulfuric, hydrochloric and nitric acids tend to be widely used [25]. Therefore, in this study, the effects of pH on pectin yield were initially investigated. As shown in Table 6 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*., [11], 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 pectin such as DE, gelling properties, ratio of galacturonic acid to rhamnose, average molecular weight and gel strength [12]. Acids are generally the strongest extracting agents with regards to the yield of extracted pectin [26, 27]. Using nitric acid at pH 1 yield of 20.36 was obtained from lemon peel at the same processing conditions.

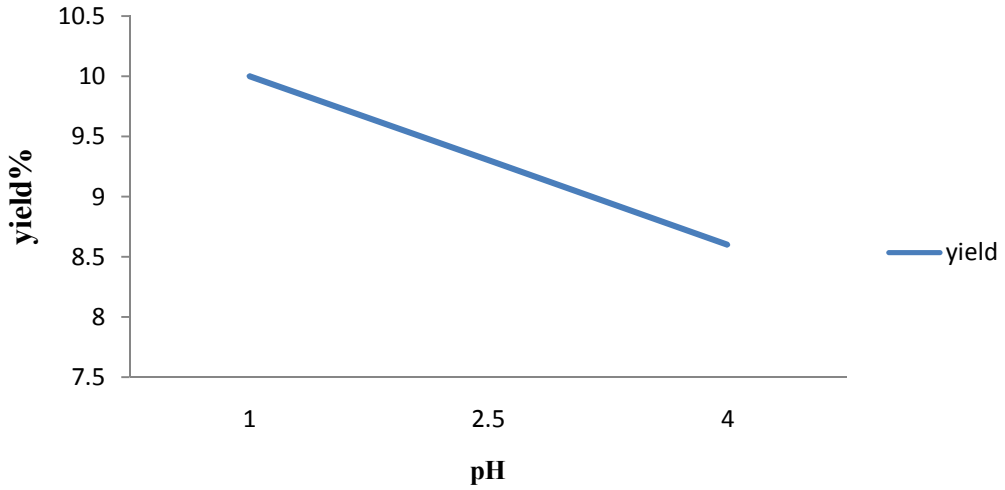
Effect of PH



(A) At temperature of 40 ^{0}C and time at 1 hour



(B) At temperature of 60 ^{0}C and time at 2 hours



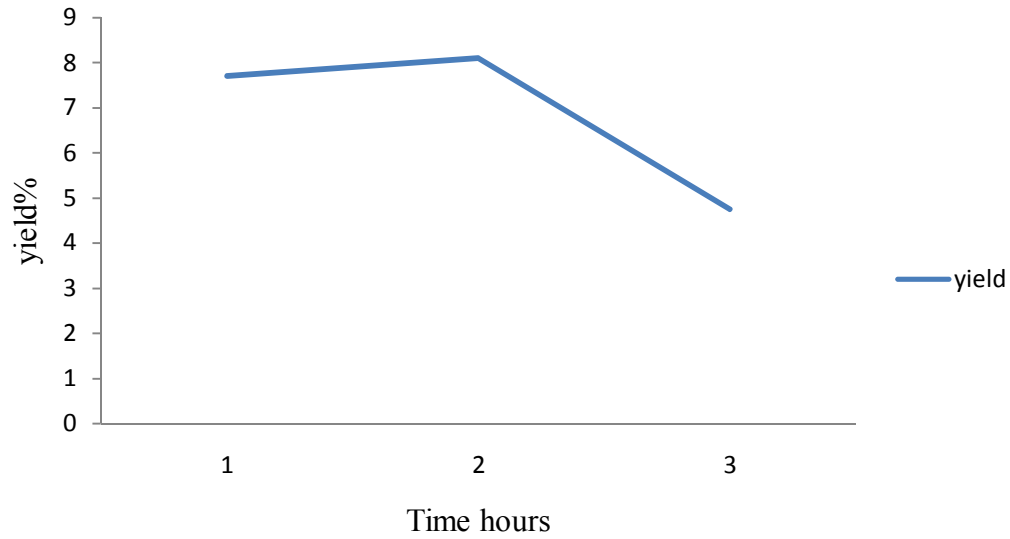
(C) At temperature of 80 ^{0}C and at time 3 hrs

FIGURES8. Pectin yield versus pH at different temperatures

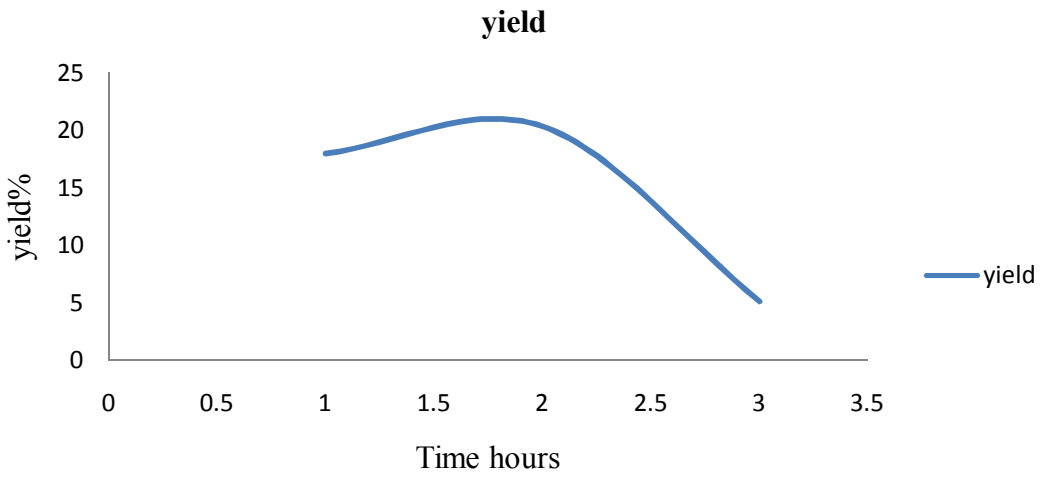
The above three fig.8 A,B&C clearly shows the change in the yield of pectin at different pH while the temperature and time kept constant. As the pH strength decreases from 1-4 pectin yield also decreases for all temperatures and times. Minimum yield was obtained for three temperatures and times at pH 4 and maximum yield was obtained at pH 1 for all temperatures and times. At pH, pectin yield was found maximum as 8.2, 20.36 and 10.0% for temperatures 40, 60, and 80°C respectively. Altaf *et al*. [2] using HCl from papaya peel observed that with increase in pH pectin yield decreased.

Effect of time on pectin yield

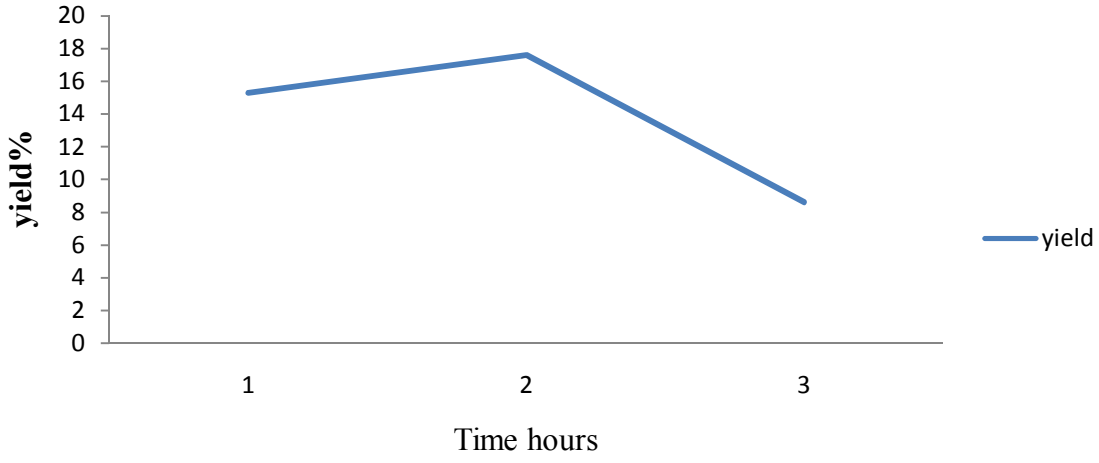
Mesbehi *et al.* [14] investigated the effect of the extraction time (3 and 4 hrs) on sugar beet pectin yield and quality. They showed that the yield attained was not significantly greater after 4 hours than 3 hours, but the pectin degradation was more pronounced. They therefore chosen the optimum time of extraction as 3 hrs.



(A) At pH 2.5 and 40 ^{0}C temperatures



(B) At pH 1 and 60 ^{0}C temperatures



(C) At pH 4 and 80 ^{0}C temperatures

FIGURES 9. Pectin yield versus temperature at various pH values

The fig. 9 A,B and C shows the change in the yield of pectin at different time while the temperature and PH is kept constant. The All figures show that the maximum yield is obtained at time of 2 hours for all pH and temperatures.

Optimization of the yield of pectin

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 second level of the third factor time 2 hr. 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 second level of the third factor time 3 hrs [1].

Physicochemical characterization of extracted pectin

Ash content

Ash content of pectin extracted from lemon peel powder varied from (3.08 -3.39%), by using nitric acid. Ranganna [20]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 lemon peel is on the appropriate range (table 2). Lower ash content was estimated from lemon peel pectin which is suggested that it can produce excellent gels [5].

Table 2: Physicochemical properties of pectin

Temperature (^{0}C)	pH	Time (hr)	Ash Content (%)	Moisture content (%)	Equivalent Weight	MeO (%)	AUA (%)	DE (%)
40 °C	1	1	3.17	8.62	384.6	3.44	65.31	29.9
60 °C	2.5	2	3.39	8.59	338.3	3.87	74	29.73
80 °C	4	3	3.08	8.61	312.5	4.3	80.76	30.23

Moisture content

The moisture content was determined by [3] using equation (3). Table 2 shows that the moisture content of pectin extracted from lemon peel was between (8.59 - 8.62%). 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. Moisture content obtained in lemon peel pectin not significantly as compared with others commercial citrus pectin. Low moisture content in pectin is required for safe storage and to prevent the progress of microorganisms which can affect the superiority of pectin because of the pectinase enzymes production [16].

Equivalent-weight

The equivalent weight of pectin extracted from lemon was between 312.5and 384.6. Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin [20, 21]. 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.* [18]. The values of equivalent weights were used for calculating the anhydrouronic acid (AUA) content and the degree of esterification. Equivalent weight of pectin indicates the ability of jell formation with high molecular weight of pectin that has better ability [23]. Extracted pectin from lemon peel waste has higher equivalent weight (table 2).

Methoxyl content

Methoxyl content is defined as the number of moles of methyl alcohol in 100mol 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 [7]. Methoxyl content of lemon peel pectin derived varies from 3.44 to 4.3% (table 2). 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 [10].

Anhydrouronic acid content

The results showed that the Anhydrouronic Acid content of pectin extracted from lemon varied from (65.31 - 80.76%). The Anhydrouronic Acid content will be higher by increasing time of extraction. A minimum value of 65% AUA for commercial pectin's 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 ej Codex 1996). However, the AUA content obtained peel powder pectin was greater than 65%. Result indicates that the extract is appropriately pure due to the possible nonappearance of proteins, starch and sugars in the precipitated pectin [17].

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 lower DE (29.73 - 30.23). Degree of esterification of pectin can be different depending on ripeness, part of fruit, botanical origin and isolation method [4]. 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 pectin's which is pectin with a DE of 58-65%.

CONCLUSION

In this research, production of pectin from locally available citrus variety namely lemon fruit has been investigated. The three processing conditions affecting the percentage of pectin yield are namely extraction temperature, pH and extraction of time has also been studied. The outputs of the experiment conducted have been analyzed by employing Design-Expert three-level-three factor general factorial design, through analysis of physicochemical characteristics. Maximum extraction yield of pectin from lemon fruit peel is found to be as follows: temperature of 60 °C, extraction time of 2 hr and pH of 1.0. Under these optimal conditions, 20.36% pectin was extracted from the lemon peel. The percentage yield

was minimum (5.1%) in sweet lime 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 5.69%. Based on the analysis of experimental results; it is found that the three process variables exhibited significant interaction effect on the yield of pectin. 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, extraction time and pH significantly affect the yield of pectin. The pectin extracted from the peels of lemon fruit exhibited medium to high degree of esterification (29.73% -30.23%). Lemon peel waste can be used as a better source of pectin which can be used for making the food products in food industries.

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