International Journal of Advanced Science and Research
ISSN: 2455-4227, Impact Factor: RJIF 5.12
www.allsciencejournal.com
Volume 1; Issue 12; December 2016; Page No. 12-15

Extraction and characterization of pectin from lemon peel

P. Alfa Ezhil Rose, D. Abilasha

Assistant professor, Department of Chemistry, Nanjil Catholic College of Arts and Science, Nedungode, Kaliakkavilai, Tamil Nadu, India.

Abstract
The present study was focused on the potential of citrus peel as a source of pectin. Pectin was extracted from lemon peel powder using nitric acid and at three different temperatures, time and pH viz (60, 70 & 80^{0}C), (30, 45 & 60 min), (1.5,2 &2.5pH) respectively. Pectin yield extracted by using citric and nitric acid as reagents medium varied from 15.8% to 67.8% and 13.8% to44.2% respectively. The best extraction condition by both the extraction reagents showed higher in yield by using citric acid at 80^{0}C, 60 min, 1.5 pH. The isolated pectin using nitric acid as reagents contained 510 equivalent weight 5.45% methoxyl content 65.4% anhydrouronic acid respectively. The degree of esterification of extracted pectin showed low methoxyl pectin. The ash and moisture content of isolated pectin were also determined. The sensory quality of the developed jelly was analyzed.

Keywords: AUA, developed jelly, equivalent weight, %DE,% methoxyl content, pectin yield, pH, reagents, time, temperature, waste utilization

1. Introduction
Lemon (Citrus Limon from Rutaceae) is one of the citrus fruits, most commonly grown tree fruit in the world. Citrus fruits are the top not only in total production, but also in economic value. 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 (Me Gready, 1996) [6].

Pectin is the methylated ester of polygalacturonic acid that contains 1, 4-linked ∝-D-galacturonic acid residues (Levigne *et al*. 2002) [4]. It is commonly found in the cell walls and middle lamallae of higher plants. These polysacchaides consist of 300-100 chains of galacturonic acid units (Yeoh *et al*. 2008) [10]. Pectin is widely used in the food industry as a thickener, emulsifier, texturizer and stabilizer. Pectin is usually added in jams and jellies as a gelling agent. It has also been as a fat substitute in spreads, ice cream and salad dressings. In terms of nutrition, pectin has been shown to lower blood cholesterol levels and low density lipoprotun cholesterol fractions, which is beneficial for human health (Liu *et al*. 2006) [5] According to the FAQ (1969) [1], pectin is considered to be a safe additive that can be taken daily. Pectin can be obtained from many sources with a variation the percentage yield. Sugar beet and sunflower head residues consist of 10 to /20% pectin (niiyamoto & Chung 1992). Other sources for pectin include cocoa husk, with about 9% of the dry weigh pectin (mollear *et al*. 2008) [8] and soyahull, with pectin contents at about 26-28% (kalapathy & proctor 2001) [3]. The yield of pectin usually depends on the extraction conditions, such as temperature, extraction time, pH, type of extraction solvents (Yeoh *et al*. 2008) [10], and drying (monsoor 2005) [9]. Before extraction begins, an alcohol- insoluble, residue is prepared to remove low molecular weight compounds, including any traces of free galacturonic acid (Happi *et al*. 2008) [2]. Pectin can be divided into two types based on the degree of esterification (DE) of the pectin: high methoxyl pectin (DE >50%) and low methoxyl pectin (DE<50%) (Meshbahi *et al*.)

1.1 Experimental Materials and Methods
Lemon were peeled and washed in order to remove dirt, dust and the residues of the pesticide spray. They were cut into small pieces, then blanching with boiling water for 5 minutes to inactivate enzyme. Then filtered by hands through two cheese cloths or muslin cloths, after which the insoluble materials (pieces) were treated in warm absolute ethanol for 30 minutes to remove oil. Then pressed under hand pressure to remove excess water. The alcohol-insoluble solids (AIS) from lemon peel pieces, thus obtained was dried at 60ºC in tray drier until the weight comes constant, then grinded and stored in tightly closed container.

1.2 Extraction of pectin
The extraction procedure was based on method given by Kratchanova M. *et al*. 5g of the peel powder was weighed and put into a 250ml conical flask, added 150 ml distilled water. Acid was added for maintaining different pH medium as reagents. Likewise for maintaining the above three pH medium, added 0.8ml,0.4ml and 0.2ml nitric acid (70% conc.) respectively. Extraction was done by hot water bath procedure. Thereafter, the mixture was heated for each different pH medium of extraction while stirred at 60, 70 and 80°C for each different time 30, 45 and 60min. The hot acid extract was filtered, three different pH medium of extraction at three of time and temperature, extraction was carried out and collected the extract separately for further experiments. The filtrate was cooled to room temperature.

1.3 Purification and Centrifugation Procedure
Pectin containing aqueous extract was coagulated by using an equal volume (1:1) of 99.1% ethanol at 4°C and was left for 3 hour. The precipitate (ethanol-insoluble fraction) formed was

recovered through centrifugation and filtration, was washed with 55% and 75% ethanol.

1.4 Percentage yield of pectin

The pectin yield was calculated using equation 1.

$$Y_{pec}(\%) = \left(\frac{p}{Bi}\right) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where, *y* pec (%) is the extracted pectin yield in percent (%), P is the amount of extracted pectin in g and Bi is the initial amount of lemon peel (5g).

1.5 Characterization of Pectin

Moisture content: 1g of sample was weighed in desiccators and was then dried in oven for 4 hour at 100°C.Then cooled over silica gel. Percent moisture observed is added (1%) to obtained agreement with the Fischer method.

Ash content: Ash content of pectin was determined by Ranganna's method (1995). Weighed 1.2g of pectin substance (sample).The sample was ignited slowly, then heat for 3-4 hour at 600 ºC. Then cooled the crucible to room temperature in a desiccators and weighed properly. The process will be repeated till constant weight is obtained.

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

W2 - Final weight of dish and ash, W1- Weight of dish, W- Weight of pectin sample.

Equivalent Weight: Equivalent weight is used for calculating the anhydrouronic acid content and degree of esterification. 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 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.

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

1.6 Methoxyl Content (MeO)

The methoxy content was determined by saponification of the pectin and titration of the liberated carboxyl groups. 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 0.25 N hydrochloric acid was added and titrated against 0.1 N NaOH to the same end point as before like in equivalent weight titration.

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

1.7 Total Anhydrouronic Acid Content (AUA)

Estimation of anhydrouronic acid content is essential to determine the purity and degree of esterification, by using equivalent weight and methoxyl content value. Total AUA of pectin was obtained by the following formula (Mohamed & Hasan, 1995).

$$\%\ 0f\ AUA = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

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

1.8 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 formula

$$\%DE = \frac{176 \times \%MeO}{31 \times \%AUA} \times 100$$

Where % MeO = Methoxyl content %
AUA=Anhydrouronic Acid Content

2. Results and Discussion

2.1 Effect of extraction reagent on pectin yield extracted from lemon peel powder (LPP) using nitric acid as reagent at different treatment combination

Pectin yield obtained from LPP using nitric acid was less as compared to using citric acid, may be due higher in molecular weight of citric acid and less depolymerisation of pectin. The percentage yield of pectin extracted by using nitric acid from LPP ranged from 13.8% to 44.2%. The percentage yield ranged of pectin at 2.0 pH was little higher than 1.5pH and 2.5pH for 30 min extraction.

By using nitric acid as reagent the yield obtained at the same pH, time and of extraction is less as compared with the yield obtained by using citric acid. In this case also too much higher or lower in pH of extraction medium was found to be less in yield.

2.2 Effect of parameters on pectin yield

The effect of extraction time, pH of solution and temperature on pectin yield extracted from LPP using nitric acid

1. Percentage yield of pectin at pH 1.5

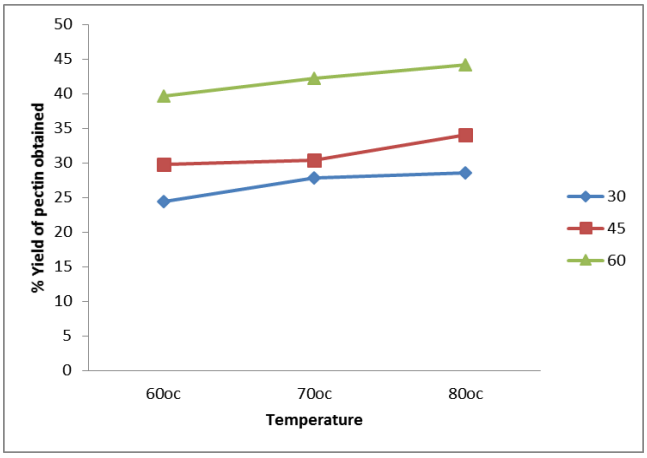

Fig 1: Effect of time and temperature on pectin yield at pH1.5 using nitric acid

The percent yield of pectin extracted from LPP using nitric acid at 1.5pH for 30min at temperature 60, 70 and 80°Care

24.4, 27.8and 28.6% respectively.At1.5 pH for 45min at temperature 60, 70 and 80°Care 29.8, 30.4 and 34% respectively. At 1.5pH for 60min at temperature 60, 70 and 80°C the % yield are 39.6, 42.2 and 44.2% respectively.
At this fixed pH, the effect of time and temperature on percent pectin yield was found to be more when extracted for 60 min. There was less increased on yield when extracted for 30 and 45 min. This is shown in fig.1, as the temperature and time increases then more in the pectin yield.

2. Percentage yield of pectin at pH 2

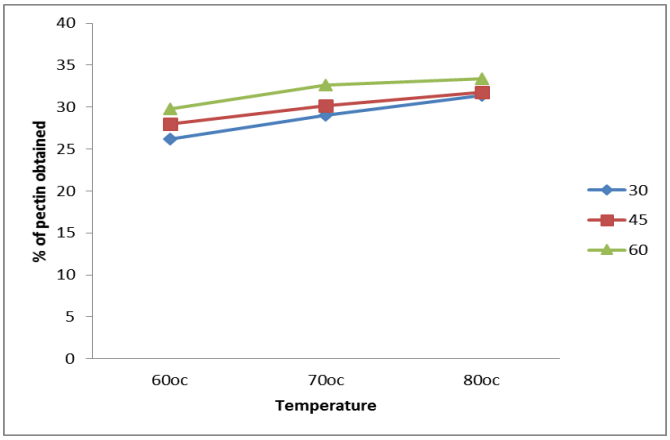

Fig 2: Effect of time and temperature yield at pH2 using nitric acid

The percentage yield of pectin at 2 pH for 30min. at temperature 60, 70 and 80°Care 26.2, 28.0 and 29.8% respectively. At 2pH for 45 min. at temperature 60, 70 and 80°C the percent pectin yield are 29, 30.2 and 32.6% respectively. Likewise at 2 pH for 60min. at temperature 60, 70 and 80°C the % yield are 31.4, 31.8 and 33.4% respectively as shown in fig.2. As the temperature increased, there was increased on pectin yield also increased. At this fixed pH, effect on pectin was more as the time period of extraction and temperature increases. For 60min of extraction there is less difference on the pectin yield at 70 and 80°C temperature.

3. Percentage yield of pectin at pH 2.5

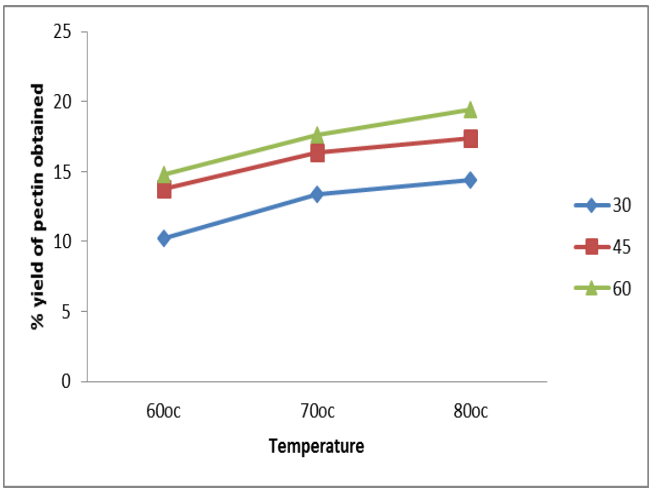

Fig 3: Effect of time and temperature on pectin yield at pH2.5 using nitric acid

The percentage yield of pectin extracted from LPP using nitric acid at 2.5 pH for 30min at temperature 60, 70 and 80°C are10.2, 13.8 and 14.8% respectively. At 2.5 pH for 45min. at temperature 60, 70 and 80°Care 13.4, 16.4 and 17.6% respectively. Likewise at 2.5 pH for 60min. at temperature 60, 70 and 80°C the % yield are 14.4, 17.4 and 19.4% respectively, as shown in fig. 3. At this fixed 2.5 pH, as the time of extraction increased, the yield was found to be more at higher temperature. But the effect of 30min time of extraction obtained low percent of pectin yield. Less time found less in pectin yield, low temperature and more time also initially increased on pectin yield to a limited level.
At this fixed pH 1.5, the effect of time and temperature on percent pectin yield was found to be more at 60 min of extracted. There was less increased on the pectin yield for 30 and 45 min of extracted.

4. Characterization of pectin

Table 1: Equivalent weight and methoxyl content using nitric acid

Normality Of NaoH	Volume of NaoH (ml)	Weight of sample	Equivalent weight	Volume of NaoH	Weight of sample	Methoxyl content	Indicator
0.1	9.8	0.5	510	8.8	0.5	5.4	Phenol red

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

$$\text{Methoxyl content\%} = \frac{\text{ml of alkali x Normality of alkali x3.1}}{\text{Weight of sample}}$$

The equivalent weight of pectin extracted from lemon peel powder (LPP) using nitric acid was found to be 510 respectively. High equivalent weight would have higher gel forming effect. The lower equivalent weight could be higher partial degradation of pectin. The increased or decreased of the equivalent weight might be also dependent upon the amount of free acid.

The methoxyl content of pectin extracted from lemon peel powder (LPP) using nitric was found to be 5.45% respectively. Methoxyl content is an important factor in determining the gel formation capacity. Methoxyl content is an important factor in controlling the setting time of pectins and the ability of the pectin to form gels.

2.3 Total Anhydrouronic acid content (AUA) using nitric acid

$$\frac{\%\, 0f\ AUA = \frac{176 \times 0.1z \times 100}{w \times 1000} + 176 \times 0.1z \times 100}{w \times 1000} = 65$$

The AUA content of pectin extracted from lemon peel powder (LPP) using nitric acid was found to be 65.4% respectively, The AUA indicates the purity of the extracted

pectin and its value should not be less than < 65% (Food Chemical Codex, 1996). In this study the highest AUA content of pectin was found using nitric acid (51.04%).

2.4 Determination of Degree of Esterification using nitric acid

$$\% \text{ D E} = \frac{176\text{x}\% \text{ MeO x } 100}{3.1\text{x}\%\text{AUA}} = 20.072\%$$

The degree of esterification of pectin extracted from lemon peel pectin (LPP) using nitric acid was found to be 20.07% respectively In this study, the pectin can be categorized as low methoxyl pectin (LMP) because it has a % DE that is lower than 50%.Degree of esterification decreased with the increase of maturity.

2.5 Ash content using nitric acid

$\% \text{ ash} = \frac{W2-W1}{W} \times 100 = 6.66\%$

The ash content of pectin extracted from lemon peel powder (LPP) using and nitric acid was found to be 6.66% respectively.

3. Conclusion

This research emphasized on pectin extraction and characterizations from lemon peel. In general, the research had been divided into three parts namely effect of reagents on pectin yield, effect on pectin yield by different parameters and characterization of pectin. The results indicated that different extractants, pH, extracting temperature and time effect on the extraction yield. The best condition were, extracting temperature at 80°C at 1.5pH for 60min and using citric acid as the extracting solvent. This gave a yield of 67.8%.

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