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Extraction and characterization of Pectin from different fruits

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Abstract
Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry. In present study pectin was extracted from the peels of three different fruits viz. Orange, Sweet lime & Papaya and comparative study of characterization done. Pectin was extracted by two different acids, such as Hydrochloric acid & Citric acid at three different temperatures (65 °C, 75 °C & 85 °C), time (30, 45 & 60 mins) and pH (2.0,. 5& 3.0). Yield of extracted pectin by using hydrochloric acid and Citric acid in orange varies from (6.0%-36.1% & 4.2%- 29% respectively), Sweet lime (3.1%-21% & 2.5%-17.3% respectively) and Papaya (4.0%-19.0% & 2.0%-16.2% respectively). The best conditions for extractions using both acids were at 85°C for 60 mins at pH 2.

Keywords: pectin, orange peel, sweet lime peel & papaya peel.

1. Introduction
Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry. It has been used successfully for many food and beverage industries as a thickening agent. Pectin also has several unique properties that have enabled it to be used as matrix for the entrapment and delivery of a variety of drugs, proteins and cell. Pectin are high molecular weight polysaccharides widely spread in plant kingdom. They can be found as an integral part of the primary cell wall and middle lamella of higher plants consisting of linear polymers of D-α (1-4) anhydrogalacturonic acid, part of the carboxyl groups of the anhydrogalacturonic acid is esterified with methanol (Wosiacki, 1977) [1]. Pectin is a purified carbohydrate product obtained from the inner portion of the rind/peels of citrus fruits. It consists chiefly of partially methoxylated polygalacturonic acid. Pectin is capable of forming gels with sugar and acid under suitable conditions. It is found almost universally in plant cell of all species suitable for use in the production of sugar jellies and industrial production of apple pomade, citrus peel and sugar beet chips, pectin extracted from various materials can be different in molecular structure (i.e. molecular weight and degree of esterification) and therefore possesses different functional properties (Joslyn,1980). It is found that pectin have several biological and physiological functions in human health. As dietary fibers pectin polysaccharides are able to regulate the lipid metabolism to reduce the absorption of glucose in the serum of diabetics and to intensify the detoxification from heavy metals. Some pectin has immune-regulatory effects in the gastric mucosal immune system through patch cell. Pectin has been used in antidiarrheal products and to lower blood lipoprotein level. Pectin also has been investigated for its ability to reduce the consequence of exposure to radiation and to inhibit prostate cancer growth. Pectin has applications in the pharmaceutical industry. Pectin favorably influences cholesterol levels in blood. It has been reported to help blood cholesterol in a wide variety of subjects and experimental conditions as comprehensively reviewed (Sriamornsak, 2001) [2]. Pectin is an interesting candidate for pharmaceutical use, e.g.as a carrier of a variety of drugs for controlled release applications. Many techniques have been used to manufacture the pectin-based delivery systems, especially inotropic gelation and gel coating. These simple techniques, together with the very safe toxicity profile, make pectin an exciting and promising excipient for the pharmaceutical industry for present and future applications. Pectin and combinations of pectin with other colloids have been used extensively to treat diarrheal disease especially in infants and

children. In the present study pectin was extracted, quantified, characterized and comparative study of pectin isolated from orange, sweet lime, papaya lime was performed.

2. Materials and methods

2.1. Sample preparation

Ripped orange, sweet lime, papaya fruits were purchased from local market, Akola (Maharashtra).The fruits were washed with warm water to remove any adherence dirt and dust and then peeled. Peels were then cut into small pieces for efficient drying and were blanched for 5 minutes to inactive the enzyme which may cause undesirable changes latter on. The peels were removed from blanching pan and were then treated with warm absolute ethanol for 25 minutes to remove oily substance form peel. The treated peels were than dried in a tray drier at 55 °C for overnight. The Dried peels were then grinded to prepare fine powder which was then used for extraction.

2.2. Extraction of pectin

Extraction of pectin was carried out by using two different acid:

1) Hydrochloric acid
2) Citric acid

250ml of distill water was added to 5 g of each fruit peel powder followed by adjustment of pH to a desired value. To maintain 2.0, 2.5, 3.0 PH of extraction medium, requires 15g, 10g, 7g, citric acid respectively. Likewise to maintain the above three pH of extraction medium, required 1.0ml, 0.8ml, 0.5ml, hydrochloric acid respectively. Then the mixture was heated for each different pH medium of extraction with stirring at 65 °C, 75 °C, and 85 °C, for each different time 30min, 45min, 60min. The hot acid extract was filtered through muslin cloth.

For each acid, three different pH medium of extraction at three different ranges of time and temperature, extractions was carried out & collected separately for further experiments. The filtrate was cooled to 4 °C of temperature. 96% ethanol was added on equal volume basis of extract, and kept for one hour the ethanol caused coagulation of pectin which was recovered by filtration. The coagulated pectin was put in petridish and kept in tray dried at 55 °C for 5 hours. The pectin obtained was then grinded for analysis (Bhat S., Sing Er., 2014) [4]

2.3. Analysis and Characterization of pectin

The yield of pectin was calculated as per the following formula (Ranganna.1995) [3]

$$Pectine\ (g/100) = \frac{Weight\ (g)\ of\ dried\ pectine}{Weight\ (g)\ dried\ pomace\ powder\ takne\ for\ extraction} \times 100 \ldots\ldots$$

2.4. Moisture content

The moisture content was calculated by the following formula (Ranganna.1995) [3]

5gm of sample in previously dried and tared dish was Weighed and placed in hot air oven for 2hours at 130 °C. The dish was removed and cooled in a desiccators and then weight.

$$Moisture\ \% = \frac{W1 \times W2}{W1 - W} \times 100 \ldots\ldots\ldots$$

W is weight of petridish (g), W1 is weight of petridish with sample (g), W2 is weigh of petridish with dried sample (g)

2.5. Ash content

It was determined as per reference of (Ranganna.1995) [3].

Weighed 1.2g of pectin substance (sample).The sample was ignited slowly and then heat for 3-4 hr at 600 ºC. Then cooled the crucible to room temperature in a desiccators and weighted properly. The process will be weighted till constant weight come and final weight will be noticed.

$$Ash\ \% = \frac{W2 - W1}{W} \times 100 \ldots\ldots\ldots$$

W2 is Final weight of dish with Ash, W1 is Weight of dish. W is Weight of sample

2.6. Equivalent weight

Equivalent weight is used for calculating the anhydro uronic 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. It was determined by Ranganna's method (1995) [3]. Pectin sample (0.5g) was weighed into a 250 ml conical flask and moistened with 5 ml ethanol; 1.0 g sodium chloride was added to the mixture followed by 100 ml distilled and few drops of phenol red indicator. Care was taken at this point to ensure that all the pectin had dissolved and that no clumping occurred at the sides of the flask before the solution was then slowly titrated (to avoid possible de-esterification) with 0.1 M NaOH to pink color at the endpoint.

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

2.7. Methoxyl content

Determination of MeO content was done by using the Ranganna's method (1995) [3]. 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 0.1N 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. It is determined by saponification of the pectin and titration of the liberated carboxyl group.

3. Result

3.1. Effect of different Acids on pectin yield

3.1.1. Effect on yield of pectin from orange, sweet lime and papaya peel using Hydrochloric Acid a Reagent for extraction:

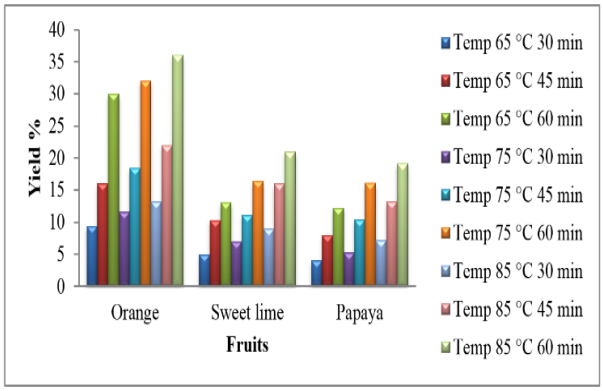

Fig 1: Effect of time and temp on pectin yield by HCL at pH 2.0

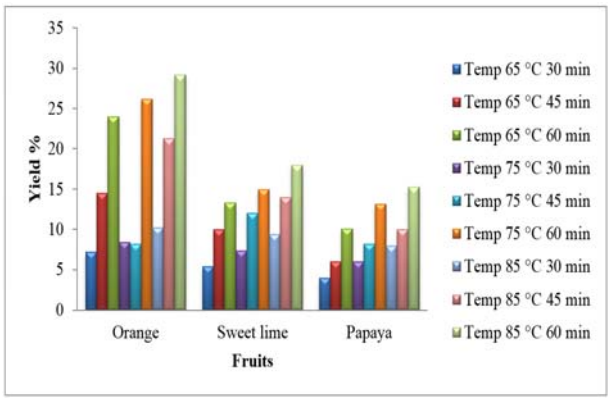

Fig 2: Effect of time and temp on pectin yield by HCL at pH 2.5.

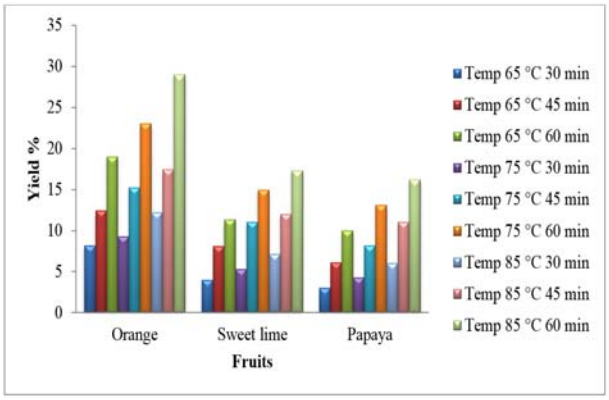

Fig 3: Effect of time and temp on pectin yield by HCL at pH 3.0

3.1.2. Effect on yield of pectin from orange, sweet lime and papaya peel using Citric acid As Reagent for Extraction.

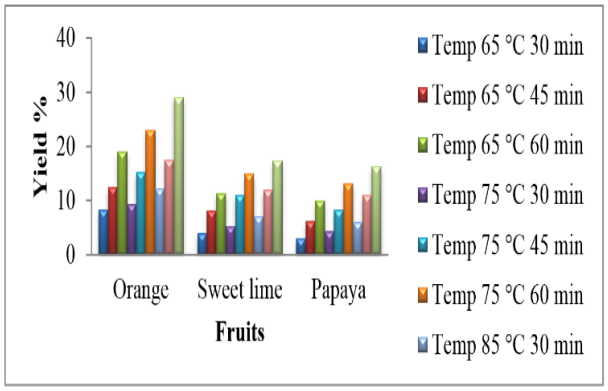

Fig 4: Effect of time and temp on pectin yield by Citric acid at pH 2.0

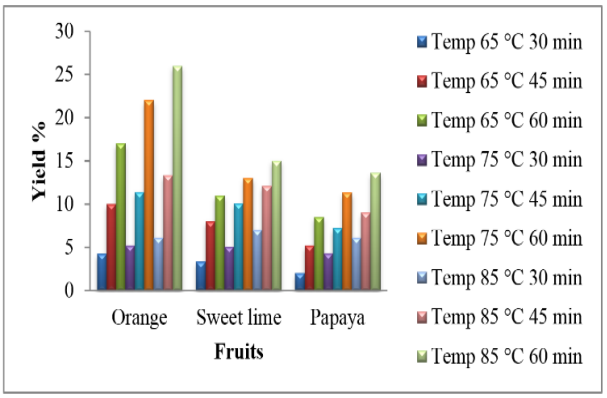

Fig 5: Effect of time and temp on pectin yield by Citric Acid at 2.5

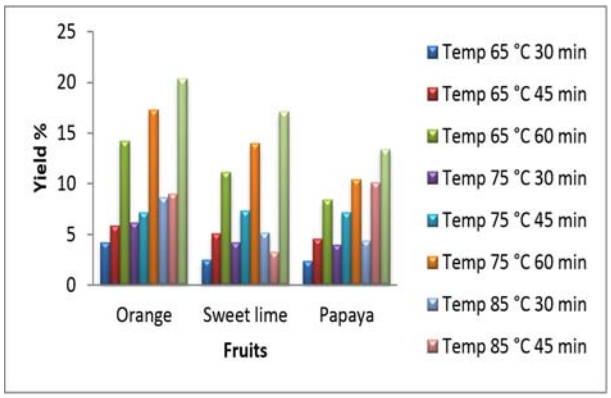

Fig 6: Effect of time and temp on pectin yield by Citric Acid at 3.0

Table 1: Showing the characterized parameters for the three samples

Characteristic / Fruit	Moisture Content (%)		Equivalent weight (%)		Methoxyl content (%)		Ash Content (%)	
	HCl	Citric Acid	HCl	Citric Acid	HCl	Citric Acid	HCl	Citric Acid
Orange	10.12	9.6	625.0	416.1	20.15	17.5	3.28	2.6
Sweet lime	9.4	9.0	555.5	333.4	15.5	12.7	3.0	2.17
Papaya	8.92	8.8	357.2	265.4	16.85	9.3	2.38	2.9

4. Discussion

The percentage yield of pectin extracted by using Hydrochloric acid from orange peel powder ranged from 6.0% to 36.1%. The percentage yield was maximum (36.1%) for orange peel powder using hydrochloric acid at treatment combination of pH 2.0 at 85 °C for 60 min. While minimum (6.0%) at treatment combination of pH 3.0 at 85 °C for 60 min. The percentage yield of pectin extracted by using hydrochloric acid from sweet lime and papaya peel powder range from 3.1% to 21%, and 4.0% to 19.1% respectively. The percentage yield was maximum (21.0%) for sweet lime and (19.1%) for papaya peel powder using hydrochloric acid at treatment combination of pH2.0at 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 pH3.0at 85 °C for 60min.

The yield of pectin extracted by using citric acid from orange, sweet lime, papaya peel powder range from (40.2% to29 %), (2.5% to17.3%), (2.0% to 16.2%) respectively. The percentage yield was maximum (29%) for orange, and (17.3%), (16.2%) in sweet lime, papaya peel powder using citric acid at treatment combination of at 2.0pH at 85 °C for 60 min, while the minimum 4.2%, 2.5%, and 2.0%. yield of pectin was obtain at treatment combination of at 3.0pH at 85 °C for 60 min.

4.1. Ash content: - Ash content of pectin extracted from orange, sweet lime, papaya, peel powder was found to be 2.6% and 2.1%, 2.4% and 3.28%, 3%, 2.9%, for hydrochloric acid and citric acid.

4.2. Moisture content: - Moisture content of pectin extracted from orange, sweet lime, papaya using hydrochloric acid and citric acid has moisture content of 10.12%, 9.6%, 9.4% and 9.0%, 8.92%, and 8.8%, respectively.

4.3. Equivalent weight: - The equivalent weight of pectin extracted from orange, sweet lime, papaya peel powder using hydrochloric acid and citric acid was found to be 625.1% and 416.1%, 555.1% and 333.4%, 357.1% and 263.2%. High equivalent weight would have higher gel forming effect. Lower equivalent weight could be higher partial degradation of pectin.

4.4. Methoxyl content: -The methoxyl content of pectin extracted from orange, sweet lime, papaya peel powder using hydrochloric acid and citric acid was found to be 20.15% and 17.5%, 15.5% and 12.7%, 10.85% and 9.3% respectively.

5. Conclusion

This research emphasized on pectin extraction, characterization and assay from three different fruits peel i.e. Orange, Sweet lime and Papaya.

The result indicated that different extraction condition such as pH, temperature, and time has effect on the extraction yield. Extraction by using Hydrochloric acid gives the best result at optimum temperature of 85 c and optimum pH at 2.0 for 60 min under such condition orange peel had given 36% yield of pectin. Which was found to be greater than sweet lime (21%) and papaya (19%).While extraction by using citric acid gives the best result at optimum temperature of 85c and optimum pH at 2.0 for 60 min under such condition orange peel had given 29% yield of pectin. Which was found to be greater than sweet lime (17.3%) and papaya (16.2%).Thus from above result we can conclude that yield of pectin by using hydrochloric acid for extraction was found to be more than citric acid? Orange peel shows high yield of pectin as compared to sweet lime and papaya.

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