EXTRACTION AND CHARACTERIZATION OF PECTIN DERIVED FROM PAPAYA (Carica papaya Linn.) PEEL

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ABSTRACT

The aim of study was to extract pectin from papaya peel and study its physico-chemical characteristics. Pectin was extracted using hydrochloric acid and citric acid, at different time, temperature and pH combinations. Extraction was done at temperatures 60°C, 70°C and 80°C for time 30 mins, 45 mins and 60 mins, and pH 2, 2.5 and 3.0 respectively. Using hydrochloric acid, the yield of pectin was higher (ranging from 2.8% - 16%) and the yield of pectin using citric acid varied from 1.9% - 9.9%. It was also observed that with decrease in pH the pectin yield increased. Maximum yield was obtained at pH 2.0 - 6.5-16% in case of HCl and 5.4-9.9% for citric acid extracted pectin. This may be due the reason that with decrease in pH the number of Hydrogen ions increases resulting in the neutralization of more carboxylic groups of pectin and higher pectin yield; and also that with increase in extraction time and temperature, the pectin yield increased. Highest yield (16%) was obtained at pH 2.0, for extraction time 60 min and extraction temperature 80°C, in case of HCl and for citric acid extracted pectin highest yield (9.9%) was obtained at pH 2.0, for 60 min time of extraction and extraction temperature 80°C. 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, which varied from 7.3%-9.67%, 4.8-7.2%, 455.1-912.17, 6.2-7.5%, 84.3-72.5% and 49.2-53.4%, respectively, using citric acid and hydrochloric acid.

Index terms: Papaya peel pectin, extraction, characterization, hydrochloric acid, citric acid, equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification.

1. INTRODUCTION

Pectin, a polysaccharide found in middle lamella of plant cell membrane, is composed of D-galacturonic acid units (Mukhidinov, 2000), linked by β-1,4 glycosidic bond. These uronic acids have carboxyl groups, which are naturally present as methyl esters, whose extent is reported as degree of esterification (DE) or methyl esterification degree (MED). Pectin is a high value functional food ingredient widely used as gelling agent and as stabilizer. It is produced commercially in 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. The high-methoxyl pectin (DE > 50%) can form gel in a condition having acid and sugar. While the low-methoxyl pectin (DE < 50%) can form gel as a complex with calcium ion. It is found that pectins have several biological and physiological functions in human nutrition and health (Yamada, 1996). As dietary fibres pectic polysaccharides are able to regulate the lipid metabolism (Groudeva et al 1997), to reduce the absorption of glucose in the serum of diabetics (Schwartz et al 1988) and to intensify the detoxification from heavy metals. Pectin can also be used in medicine as a supplement to lower cholesterol and increase immunity or as dentistry adhesive. It has been used potentially as a carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dose form.

Carica papaya plant itself has medicinal value. Different parts including leaves, seeds, latex and fruit are widely used for various purposes. C. Papaya has a wide variety of medicinal properties including anticancer, antiviral, anti-inflammatory, antimicrobial, antidiabetic, antihypertensive, wound healing activity, free radical scavenging activity and increase in thrombocyte count, etc (Sudhakar and Vidhyaram, 2014).

2. MATERIALS AND METHODS

2.1 PROCUREMENT OF SAMPLE

The papaya fruits were obtained from local market, Mahewa, Naini, Allahabad. The fruits were washed with water to remove any adhered impurities such as dirt or dust particles. The fruits were peeled and the peels were then chopped for efficient drying. These peels were then utilised for pectin extraction using hydrochloric acid and citric acid- after several pretreatments like blanching (for 2-3 mins at 95°C, to inactivate enzymes, followed by cooling), treatment with ethanol (96%), drying at 50°C in a tray drier (overnight) and then grinding to obtain fine powder. Prolonged boiling during blanching must be avoided otherwise pectin may undergo thermal degradation.
2.2 EXTRACTION

Method for extraction of pectin consisted of adding 250 ml of water to 5g pectin peel powder in a beaker and addition of 0.5N HCl or citric acid (to adjust pH) and then heating the mixture to 60°C, 70°C and 80°C for 30, 45 and 60 mins, respectively, in a hot water bath. Then the solution was filtered through muslin cloth. The pectin extract obtained from different extractions, after cooling to room temperature, was precipitated with 96% ethanol (alcoholic precipitation) and kept for an hour. Filtration was again done for separation of coagulated pectin. The coagulated pectin was then kept for drying in oven at 50°C. After drying the pectin was ground. The dried pectin obtained from both the methods of extraction was packed in food grade polythene pouches and stored in a cool dry place until further analysis. For optimization of pretreatment of papaya peel, acid extraction followed by alcoholic precipitation method was followed.

3. PHYSICO-CHEMICAL ANALYSIS

The extracted pectin was analyzed for yield, moisture, ash, equivalent weight and methoxyl content.

3.1 PECTIN YIELD

The yield was calculated using following formula

\[ \text{Pectin (g/100g)} = \frac{\text{Weight (g) of dried pectin}}{\text{Weight (g) dried pomace powder taken for extraction}} \times 100 \]

3.2 MOISTURE AND ASH CONTENT

Moisture content was determined according to the Association of Analytical Chemists (AOAC) method (AOAC, 1990). The moisture content was determined by oven drying method. 5g sample was taken in a pre-weighed petridish. Then, the sample was placed into an oven at 130°C for 2 h. Lastly, the sample was allowed to cool down in desiccators and weighed again to get the dry sample weight.

3.3 ASH CONTENT

Ash content was also determined according to the Association of Analytical Chemists (AOAC) method (AOAC, 1990).

For the ash content determination, porcelain crucibles were initially dried in muffle furnace at 600°C for 3 h and then were put into desiccators for cooling. 5 g of sample then was put into that porcelain crucibles and put into the muffle furnace at 600°C for 3 h. Then it was allowed to cool down in desiccators before it was weighed.

3.4 EQUIVALENT WEIGHT

Equivalent weight was determined by the standard methods of Owens et al. (1952). Equivalent weight was done by weighing 0.5 g pectin (moisture free) in a 250 ml conical flask, moistened with 5 ml ethanol, added 1 g of sodium chloride to sharpen the end point, added 100 ml of deionised water and 6 drops of phenol red indicator. The pectin substances were stirred rapidly to dissolve, and then titrated slowly with 0.1N NaOH until the colour of the indicator changed (pH 7.5) and persisted for at least 30 s. The neutralised solution was saved for methoxyl determination.

Equivalent weight of the pectin is expressed as under:

\[ \text{Equivalent Weight} = \frac{\text{Weight of sample} \times 1000}{\text{ml of alkali} \times \text{Normality of alkali}} \]

3.5 METHOXYL CONTENT

Methoxyl (MeO) content was determined by adding 25 ml of 0.25 N NaOH to the neutral solution, mixing thoroughly, and allowed to stand for 30 min at room temperature in a stopper flask. 25 ml of 0.25 N HCl was then added and titrated with 0.1 N NaOH to the same end point as before. Methoxyl content was calculated as per expression:

\[ \% \text{Methoxyl Content} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample}} \]

4. RESULTS AND DISCUSSION

4.1 PECTIN YIELD

4.1.1 Effect of Different Acids on Pectin Yield

The yield of the papaya peel pectin expressed as dry weight of the extract varied from 2.8% - 16% and that using citric acid varied from 1.9% - 9.9%. The highest percentage of pectin (16%) obtained was on extraction with hydrochloric acid at pH 2.0, temperature 80°C and with 60 min extraction time. And the lowest percentage of pectin (1.9%) obtained was on extraction with citric acid at pH 3.0, temperature 60°C and with 30 min extraction time.

The same results were obtained by Banu et al. (2012). They compared the pectin yield from different fruits using different acids and observed that pectin yield was highest when hydrochloric acid was used for extraction.

4.1.2 Effect of pH on pectin yield

It was observed that with increase in pH the pectin yield decreased. At pH 2, the yield was higher as compared to that at pH 2.5 and pH 3. At pH 2, while using hydrochloric acid for extraction, the pectin yield varied from 6.5% to 16%, at pH 2.5, the yield varied from 4.9% to 13.3% and at pH 3, the yield varied from 2.8% to 9.4%.

Similarly when citric acid was used for extraction the pectin yield at pH 2 varied from 5.4% to 9.9% at pH 2.5 the yield varied from 5.2% to 7.6% and at pH 3 the yield varied from 1.9% to 4.5%.

4.1.3 Effect of time and temperature on pectin yield using hydrochloric acid for extraction

It was observed that with increase in both temperature and the extraction time, the pectin yield increased. At pH 2, extraction time 30 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 6.5%, 8.2% and 9.2% respectively. For extraction time 45 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 13.0%, 13.7% and 14.5% respectively and for extraction time 60 min and temperatures 60°C, 70°C and 80°C, the
pectin yield was 15.2%, 15.7% and 16.0% respectively.

At pH 2.5, extraction time 30 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 4.9%, 6.3% and 6.8% respectively. For extraction time 45 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 7.7%, 9.7% and 11.2% respectively and for extraction time 60 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 11.6%, 12.8% and 13.3% respectively.

At pH 3, extraction time 30 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 2.8%, 4.3% and 4.7% respectively. For extraction time 45 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 5.0%, 5.7% and 6.0% respectively and for extraction time 60 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 7.6%, 9.0% and 9.4% respectively.

4.1.4 Effect of time and temperature on pectin yield using citric acid for extraction

It was observed that with increase in both extraction time and temperature, the pectin yield increased. The pectin yield (using citric acid) at pH 2.0, extraction time 30 min and temperatures 60°C, 70°C and 80°C was 5.4%, 6.2% and 6.5% respectively, for time 45 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 6.9%, 7.0% and 7.8% respectively and for extraction time 60 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 9.3%, 9.7% and 9.9% respectively.

The pectin yield (using citric acid) at pH 2.5, time 30 min and temperatures 60°C, 70°C and 80°C was 5.2%, 5.7% and 6.1% respectively. For extraction time 45 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 6.5%, 6.8% and 7.3% respectively and for extraction time 60 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 7.0%, 7.2% and 7.6% respectively.

The pectin yield (using citric acid) at pH 3.0, extraction time 30 min and temperatures 60°C, 70°C and 80°C was 1.9%, 2.5% and 3.0% respectively. At extraction time 45 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 2.7%, 3.7% and 3.9% respectively and at extraction time 60 min and temperatures 60°C, 70°C and 80°C, the pectin yield was 4.0%, 4.3% and 4.5% respectively.
The type of pectin in this research was categorized as low methoxyl pectin because the ester groups were less than 50%.

4.2 MOISTURE CONTENT

The moisture content of pectin extracted using hydrochloric acid was 6.7% and that extracted using citric acid was 7.3%. Hence the pectin extracted using citric acid as reagent is more hygroscopic. The difference in hygroscopic nature of pectins is due to different degrees of esterification, as reported by Sharma et al. (2014). Based on the quality standards of commercial pectin, all of pectin is produced to meet the standards not far above 12%.

4.3 ASH CONTENT

The ash content of pectin extracted using HCl and Citric acid was 7.2% and 4.8% respectively. Papaya peel pectin obtained in this research was categorized as pectin with high ash content. 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.

4.4 EQUIVALENT WEIGHT

The equivalent weight of pectin extracted using HCl and Citric acid was 912.17 and 455.1 respectively. Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin (Ranganna, 1986). Pectin produced at low pH has higher equivalent weight, because low pH can cause polymerization of pectin into a longer chain, and inturn decreases the free acid content, reported by Rouse (1977).

4.5 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 papaya peel pectin derived using HCl was 7.5% and of that derived using citric acid was 6.2%. The type of pectin in this research was categorized as low methoxyl pectin because the ester groups were less than 50%.

4.6 ANHYDROURONIC ACID CONTENT

The results showed that the Anhydrouronic Acid content of pectin extracted using Hydrochloric acid was less (70.5%) as compared to that of pectin extracted using Citric Acid (84.3%). 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.

4.7 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 using hydrochloric acid has higher DE (53.4%) as compared to that extracted using citric acid (49.2%). Degree of esterification of papaya pectin can be different depending on ripeness, part of fruit, botanical origin and isolation method (Bonrood et al. 2005). Unripe papaya has higher degree of esterification. The type of pectin in this research was categorised as low methoxyl pectin because the ester groups were less than 50%.

5. CONCLUSION

In the study, different extraction conditions were used to extract the pectin from papaya peel. The extraction conditions had major impact on the extraction yields of pectin. It was observed that the pectin yield was high using hydrochloric acid for extraction as compared to that using citric acid. And it was also concluded that with increase in extraction time and temperature, the pectin yield increases. However, increase in pH decreases the pectin yield.

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