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Research Article

Extraction and characterization of pectin derived from underutilized papaya seeds as a value-added product

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Abstract
Food processing industries generate a massive amount of biowastes, which causes major environmental issues. High-level marketable bioproducts can be extracted from these biowastes as value-added products. One such value-added product is pectin. Papaya fruit is one of the tropical fruits that is utilized the most to produce a greater number of processed foods in the food processing industries. Papaya seeds are one of the underutilized parts of papaya and have potential commercial value-added products. The present study aims to extract pectin from papaya seed waste using the hot water extraction technique. Furthermore, one factor at a time (OFAT) was used to find the optimum process conditions for the high extraction of pectin. The parameters considered were liquid–solid ratio (5-50 ml/g), sample weight (5-25 g), extraction time (15-90 min), temperature (50-100°C) and pH (1-3). A high yield of pectin (8.655%) was obtained at a liquid–solid ratio of 25 mL/g, sample weight of 20 g, extraction time of 60 min at 80°C, pH of 1.5 and precipitation with ethanol. Proximate analysis was performed for the papaya seeds that had moisture (82.10%), ash (1.76%), protein (1.52%), fat (1.42%) and carbohydrate (13.20%), and the pectin extracted from papaya seeds were found to have moisture (7.8%), ash (7.6%), protein (2.2%), fat (2.1%) and carbohydrate (80.3%). Pectin was characterized with gas chromatography for its methoxy content, which was found to be 9.216%. The current investigation found that pectin obtained from papaya seeds had low methoxy pectin, which has commercial applications in the jam and jelly industries.

Keywords: Gas chromatography, Methoxy content, One factor at a time, Pectin, Proximate Analysis

INTRODUCTION

Over the past decade, awareness of the environmental conservation and sustainability of resources has been growing. As a result, the management of natural resources is gaining importance. Highly perishable biowastes are generated by fruit processing industries that lead to the dumping of solid waste and steering to pollution of the environment (Minjares-Fuentes *et al*., 2014). Pectin is a highly valued functional ingredient that is widely used as a gelling agent in jellies and jams, as a thickener in ketchup and sauces, and as an emulsifying agent in acidic milk drinks (Madhav, A. and Pushpalatha.,2006). Pectin is made up of complex polysaccharides that have repeated units of D-galacturonic acid linked by α-1,4 linkages. Pectin is usually present in the cell wall and middle lamella of plant tissues. It is present in different quantities in fruit cell walls, stem cell walls and leaf cell walls of the plant (Yeoh, S *et al*., 2008). The gelling capacity of pectin depends on the methoxyl content. Based on this, it is categorized into high and low methoxy pectin. The degree of esterification (DE) helps to differentiate pectin. The value of DE in commercial HM pectin is greater than 50% and it is less than 50% for LM pectin(Leong *et al*., 2016). In the formulation of low-calorie jams, low methoxyl pectin is a suitable gelling agent owing to its ability to gel in the absence of sugar (Picot-Allain *et al*., 2020). These pectins are widely used as stabilizers, texturizers, emulsifiers, and coloring agents and have a food protecting capacity that finds numerous applications in the food industry (Lara-Espinoza *et al*., 2018).

Pectin is very often present in fruits such as apples, citrus fruits, papaya, guavas, and pears, among which apples or citrus peels are the two most commonly used fruit sources for the production of commercial pectin. Even though pectin exists in most plant tissues, industrial pectin production has a limited number of sources (Boukroufa *et al*., 2015). Tropical fruits constitute a large quantity of rind or skin and seed, which are considered to be waste. Papaya is a tropical fruit that has a high content of vitamins and enzymes, such as papain and chymopapain (Vij and Prashar, 2015). This tropical fruit comprises skin (10-20%) and seeds (10-20%), which are waste(Parni, B. and Verma., 2014), but this waste attracts the attention of researchers due to its health benefits. Papaya peels are utilized for applications such as flour, protease enzyme and pectin (Chaiwut *et al*., 2010; dos Santos *et al*., 2014 ; Koubala *et al*., 2014 and Pathak *et al*., 2019) . Papaya seeds are also good sources of value-added products such as pectin, which is underutilized.

Pectin is additionally utilized in the preparation of an assortment of items, including edible and biodegradable films, adhesives, paper substitutes, foams and plasticizers, surface modifiers for clinical devices, materials for biomedical implantation, and drug conveyance (Roy *et al*., 2017). This study aims to utilize underutilized papaya seeds for the extraction of pectin using conventional hot water extraction to screen the process parameters using OFAT to maximize pectin yield. Proximate analysis of pectin extracted from papaya seeds was also conducted to understand its composition.

MATERIALS AND METHODS

Sample preparation

Papaya seeds were procured from local fruit shops in Coimbatore, Tamilnadu. The seeds were washed to remove the adhered impurities under running tap water. Then, the seeds were allowed to dry the excess moisture.

Proximate analysis

Proximate analysis was performed for both papaya seeds and pectin extracted from them. The ash, moisture, crude protein, crude fat, and crude carbohydrate contents were estimated using proximate analysis following AOAC (Association of Official Analytical Chemists methods), and all experiments were conducted in triplicate.

Moisture

The moisture content of the sample was measured using the oven-dry method of (AOAC., 2000). The sample was dried in the oven until a constant weight was obtained. The percentage weight of moisture was calculated by the formula.

$$\text{Moisture (\%)} = \left(1 - \frac{\text{weight of dry sample}}{\text{Weight of wet sample}}\right) \times 100$$

Eq. 1

Fat

The fat content in the sample was determined by the Soxhlet extraction method (Noureddini and Byun.,2010). The extraction solvent used was petroleum ether. The percentage of fat was obtained from the following formula:

$$Fat\ (\%) = \frac{weight\ of\ fat}{Weight\ of\ sample} \times 100$$

Eq. 2

Protein

The total protein content of the sample was estimated by the Kjeldahl method (AOAC, 2000). A conversion factor of 4.4 for nitrogen to a protein was used for the estimation of protein present in the sample.

Ash

The ash content of the sample was determined by the dry ashing method according to AOAC. (2000). The samples were incinerated in a muffle furnace at 550°C. The ash was cooled and weighed to determine the ash content. The formula provided below was used to calculate the ash percentage:

$$Ash\ (\%) = \frac{weight\ of\ ash}{Weight\ of\ sample} \times 100$$

Eq. 3

Carbohydrate

The carbohydrate content in the sample was calculated by the difference method. The formula to calculate carbohydrate content is provided below:

Carbohydrate (%) = 100- (%Ash + % Moisture + % Protein + % Fat) Eq. 4

Extraction of pectin

Extraction of pectin proceeded with certain changes in the method given by (Kliemann *et al*.,2009). Then, 100 ml of citric acid solution at pH 1 was added to 20 g papaya seeds, and the extraction was performed at a temperature of 80°C for 60 min in a water bath. The extract obtained after extraction was filtered, cooled at 25°C, and centrifuged at 8000 rpm for 20 min. The pectin extracted was precipitated with 96% ethanol (Bagherian *et al*., 2011) and filtered and washed with 70% acidified ethanol and 70% ethanol twice. The extracted pectin was dried at a temperature of 50°C for 24 h and stored at 40°C.

The pectin yield was calculated with the following formula:

$$Pectin\,Yield\,(\%) = \frac{weight\ of\ extracted\ pectin}{Weight\ of\ dried\ sample} \times 100$$

Eq. 5

Experimental design

One factor at a time was employed to choose the best process parameter levels considering pectin yield as a response, where one process parameter was kept as a variable and the other parameters were kept as constants. The process parameters considered were the liquid-to-solid ratio (5-50 ml/g), sample weight (5-25 g), extraction time (15-90 min), temperature (50-100°C), pH (1-3), and precipitation solvent (ethanol, isopropyl alcohol, and AlCl3). The experiments were carried out in triplicate to evaluate the experimental errors (Rahmani *et al*., 2020).

Methoxyl content

The methoxyl content was found using gas chromatography (Shimadzu 2010) with an RTX 2330 column with a flame ionization detector. A column temperature of 200°C was maintained during injection and 250°C during detection. Nitrogen at a rate of 40 mL/min was used as the carrier gas. One microliter of the sample was injected into the column, and methanol was used as a reference. The formula for percentage methoxy content is given below:

% CH3O = % CH3OH X 0.969 Eq. 6

where 0.969 is the conversion factor for methanol to methoxy (O-CH3)

RESULTS AND DISCUSSION

Yield of extracted pectin

The present study on pectin from papaya seeds using hot water extraction gave a maximum yield of 8.655% of pectin extracted at 60 min, 80°C, and a pH of 1.5 with ethanol as the precipitation solvent from the seeds of papaya. The organic acid used for pH adjustment was citric acid in consideration of environmental safety and cleanliness. The low hydrolyzing capacity of the organic acid was due to the lower dissociation constant that led pectin to bring about proton catalyzed depolymerization as reported by De Oliveira and Vitória (2011). The pectin was extracted from papaya seeds by hot water extraction (Fig 1).

Effect of liquid-to-solid ratio

In the pectin extraction process, the liquid to solid ratio was employed in the range of (5-50 mL/g). The optimum liquid-to-solid ratio was found to be 25 mL/g (Fig 2). This can be due to the direct interaction between plant material and solvent. When the ratio was increased to approximately 25 mL/g, the yield of pectin decreased. This was due to the degradation of pectin when the solvent volume increased the solubility of the target compound. The current ratio of 5-50 mL/g with maximum yield (32.4%) is in accordance with the previously reported liquid to the solid ratio between 10 - 20 mL/g with a maximum yield of 14.48% pectin as per Moorthy *et al*.(2017).

Effect of extraction time

In the current study, the extraction time was between 15-90 min. The maximum yield was obtained at 60 min (Fig 3). The pectin yield decreased beyond the optimum time due to exposure to excessive heating, which led to alterations in the structure and disintegration of the carbohydrate pectin, as reported by Xu *et al*.(2014). Prolonged exposure of pectin to the heating process may affect the industrial production of pectin. This may lead to a decrease in production.

Effect of sample weight

The sample weight was varied from 5-25 g. The maximum yield of pectin was obtained when 20 g of sample was used in the extraction, as shown in Fig 4. The pectin yield decreased when the weight of the sample increased. This is due to the decrease in contact of solvent with the seeds. When the weight of the sample

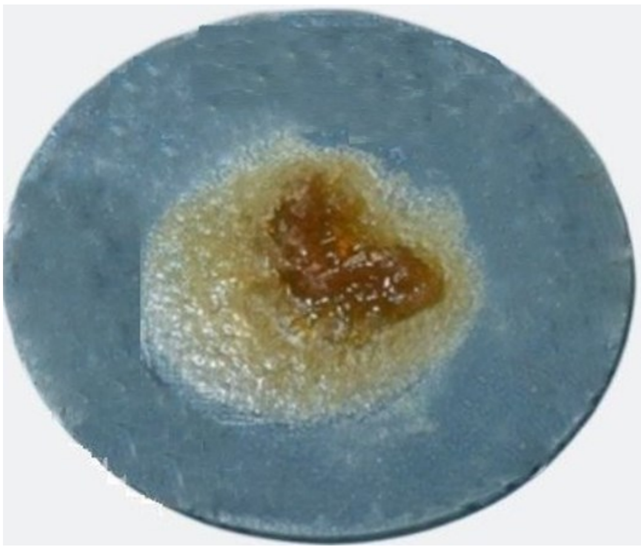

Fig. 1. *Pectin extracted from papaya seeds by the hot water extraction method*

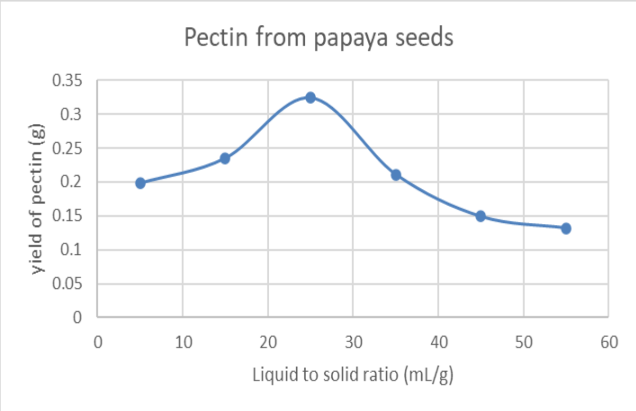

Fig. 2. *Showing liquid-to-solid ratio vs yield of pectin*

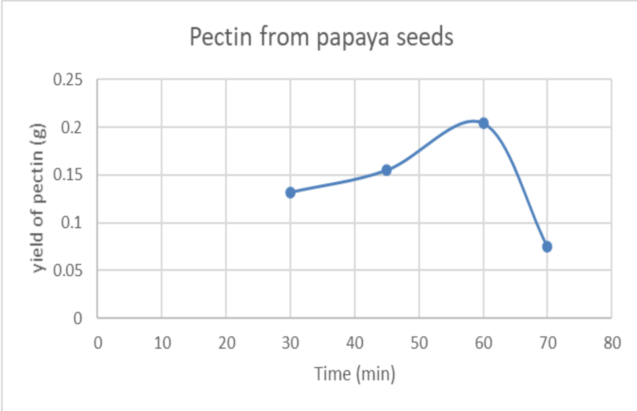

Fig. 3. *Showing time vs yield of pectin*

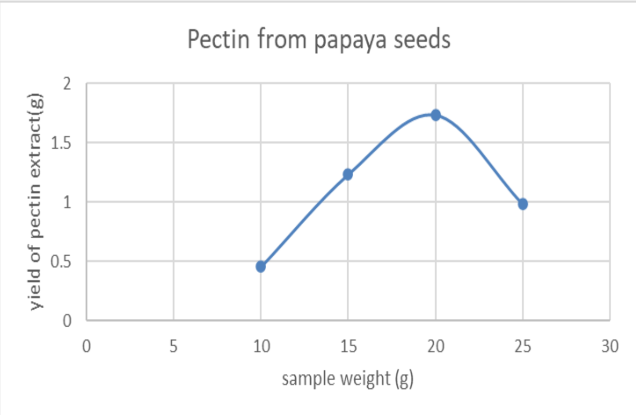

Fig. 4. *Showing sample weight vs yield of pectin*

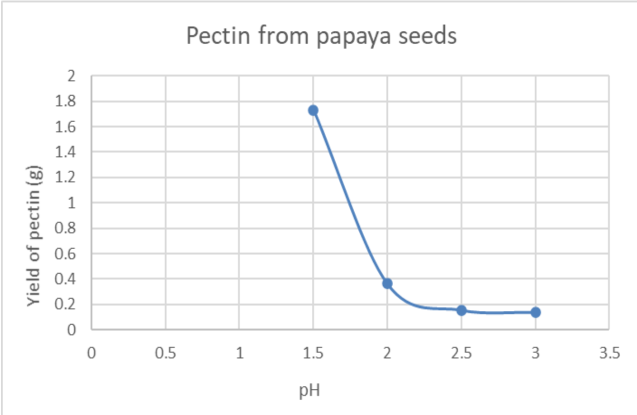

Fig. 5. *Showing pH vs yield of pectin*

increases, the interaction of the target compound with the solvent decreases. The decrease in the pectin yield with the augmentation of sample weight to solvent is not in agreement with the mass transfer principle as the concentration gradient, which is said to be higher when the sample weight to solvent is less, as reported by Moorthy *et al*. (2017).

Effect of pH

Similarly, the pH was varied from 1-3 to obtain the maximum yield of pectin. It was found in this study that at pH 1.5, the yield of pectin was maximum, as shown in Fig 5. The pectin yield decreased gradually when it reached a pH of 3. At this pH, hydrogen bonding-mediated acid-induced gelation occurred, due to which there was a decrease in pectin yield. The volume of weak acid to reduce the pH is a major parameter of concern when the process needs to be scaled up to industry level. It was found that the pH of the papaya seed boiled water in hot water extraction was around 4-5. For further reduction of pH only lower volume of weak acid was consumed.so the volume of weak acid required will not affect the industrial production of pectin. Moreover, citric acid is used in this study for pH reduction, which has biological origin and safe to be used in food and pharmaceutical applications. From previous studies, it is clear that when the pH decreases, the yield of pectin increases according to Moorthy *et al*. (2015).

Effect of temperature

A temperature range of 50-100°C was employed for pectin extraction. At 80°C, the yield of pectin was maximum, as shown in Fig 6. There was a decrease in the pectin yield as the temperature increased. The breakdown of the cell wall of the plant increased pectin release. The thermal increase thereby decreases pectin release. When the temperature reaches 80°C, there is a decrease in the pectin yield as per Ismail *et al*.(2012).

Proximate analysis of papaya seed and extracted pectin

Proximate analysis of papaya seed and extracted pectin, viz., the percentage yield of ash, moisture, crude protein, crude fat, and carbohydrate, are tabulated in Table 1. It was found that the maximum carbohydrate content present in papaya seeds is contributed by pectin. The proximate analysis provides information about various functional properties of the food contributed by the macronutrient constituents of the food (Ooi *et al*., 2012).

Methoxy content and its effect

From the GC, the methoxy content of pectin was found

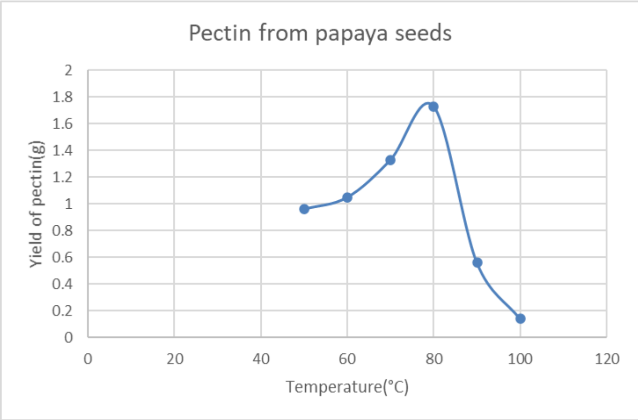

Fig. 6. *Showing temperature vs yield of pectin*

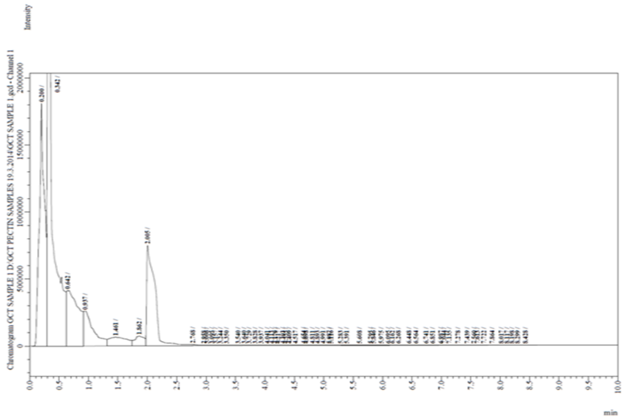

Fig. 7. *Chromatogram of pectin sample showing the methoxy content*

Table 1. Proximate analysis of papaya seed and extracted pectin

	Papaya seed	Extracted Pectin
Moisture (%)	82.10	7.8
Ash (%)	1.76	7.6
Protein (%)	1.52	2.2
Fat (%)	1.42	2.1
Total carbohydrate (%)	13.20	80.3

to be 9.216%. Methylated pectin is saponified with alkali to produce methanol and pectin salt. The concentration of methanol in the reaction solution determined by GC using an FID detector compared to the reference methanol standard is shown in Fig. 7. Pectin is made up of various sugars. These sugar moieties have a free hydroxyl group that can form methyl groups, known as methylation according to Ismail *et al*.(2012). In general, when the methoxy content of pectin is below 50%, pectin is referred to as low methoxy pectin. In this study, the methoxy content of pectin (9.216%) from papaya seeds was found to be below 50%, so pectin was categorized as low methoxy pectin. The methoxy value represents the pectin distribution capacity in water; the gel capacity of a high methoxy value may suggest strong cohesive and adhesive forces, which might be inferred from the increase in firmness of the food products, as reported by Mugampoza *et al*. (2020). Thus, low-methoxy pectin can be used in food products that do not require firmness in texture; thus, it is used as a fat substitute in baked goods and as a gelling agent, thickening agent and stabilizer.

Conclusion

From the current study, pectin with a maximum yield of 8.655% was extracted by the hot water extraction method with optimum process conditions of pH 1.5, temperature 80°C, time 60 min, and the liquid-to-solid ratio of 25 mL/g with ethanol precipitation. The methoxy content of pectin was found to be 9.216%, which is a low methoxy content in nature. Low-methoxyl pectin has been used in the food industry to create low-sugar jams because it does not require high sugar levels to gel. In the present diabetic world, low sugar jams are in demand. Most molecular recipes look for low-methoxy pectin. The extracted pectin can be developed into commercial pectin with suitable food application when further characterization is performed. This current study has led to finding other alternative undiscovered pectin sources that can stand equal to the production cost and properties of commercial pectin. To the finest of our knowledge, this is the first study on pectin extraction from papaya seeds, which is an underutilized biowaste. Thus, it is concluded that papaya seed waste can be a highly promising feedstock for pectin production in the near future.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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