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Extraction and Characterization of Pectin from Selected Fruit Wastes

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ABSTRACT

Pectin is widely used as a gelling agent, thickening agent, stabilizer, and an emulsifier in the food industry. The inedible portions like peels and cores of commercially available fruits of *nasnaran* (*Citrus madurensis* L.), *starfruit* (*Averrhoa carambola* L.), soursop (*Annona muricata* L.), avocado (*Persea americana*), and *nelli* (*Phyllanthus emblica* L.) are disposed as waste. This study thus investigated the potential of extracting pectin from above fruit wastes. Pectin was extracted by acid extraction. Only *Nasnaran*, soursop and *starfruit* yielded pectin. Moisture, ash, solubility, colour, equivalent weight, methoxyl content, anhydrouronic acid content (AUA), degree of esterification (DE) were measured in extracted pectin. Micrograph of extracted pectin was obtained by electron microscopy. Data was analyzed using ANOVA in SAS programme. Moisture and ash content of all treatments were within the accepted level. Lowest ($p<0.05$) ash (5.85±0.29%) and moisture (9.49±0.15%) contents were obtained from *nasnaran* where accepted maximum levels are 10% and 12% respectively. Pectin extracted from *starfruit* showed the highest equivalent weight (653.22±17.02) and the lowest methoxyl content (3.51±0.36%) indicating better gelling ability where acceptable methoxyl content is <7%. *Nasnaran* resulted in the highest (91.92±9.63%) AUA with highest purity (AUA >65%). DE of all samples varied from 42.42±2.11 to 48.36±3.63, thus all pectins were rated as low methoxyl pectin. *Nasnaran* pectin resulted highest solubility in both cold and hot water while soursop pectin had the lowest solubility. Pectin from *nasnaran* showed higher possibility for extracting good quality pectin and *starfruit* pectin had the better gel-forming ability.

1. Introduction

Pectin is a complex polysaccharide found in primary cell wall and middle lamella of higher plants [1]. They consist of mainly galacturonic acid units linked by α-1, 4 linkages [2]. Depending on the percentage of galacturonic acid residues that are esterified with methanol, extracted pectin can be categorized into two categories. When the degree of methoxylation (DM) is greater than 50%, it is considered as high methoxyl pectin and DM below 50% is considered as low methoxyl pectin [3].

Pectin is considered as an important dietary fiber that can reduce the risk of developing cardiovascular diseases, insulin resistant, type II diabetes along with certain types of cancers [4]. Other than medicinal benefits pectin is widely used in textiles, food industries, pharmaceuticals and other products as well [5]. Pectin is a natural polysaccharide widely used in the food industry as a gelling agent, stabilizer, and thickener for jams and jellies and also used in fruit drink concentrates, fruit juices, desserts and fermented dairy products [6].

Conventional pectin extraction is done by acid extraction method using HCl, HNO_3, H_2SO_4 and citric acids [7]. Commercially, pectin extraction is primarily performed using citrus peel and apple pomace. However, there are also alternative

sources available, including sugar beets, banana peel, mango peel, papaya peel, and sunflower heads. [3].

Fruits are widely consumed in the world due to its nutrients like vitamins and a variety of applications like fruit juices, jams and beverages.

Nelli (Phyllanthus emblica L.), Starfruit (Averrhoa carambola L.), Soursop (Annona muricata L.), Avocado (Persea americana), and Nasnaran (Citrus madurensis L.) are widely available fruits in Sri Lanka. However, in commercial settings, their peels and cores are often disposed of without being utilized.These are consumed widely as fresh fruits and also used to prepare products like beverages, wine, jellies, jam, fruit-butter preserves, and puree. Pectin extraction from these fruit wastes allows for the utilization of by-products that would otherwise go to waste. By extracting pectin from these waste sources, valuable functional ingredients can be obtained. Pectin derived from these fruits may also possess potential health benefits due to the presence of bioactive compounds and antioxidants. For example, Nelli, Soursop, and Avocado are known for their high content of phytochemicals and vitamins, which contribute to various health-promoting properties. Currently, pectin is primarily extracted from citrus fruits, such as oranges and lemons. However, diversifying pectin sources to include Nelli, Starfruit, Soursop, Avocado, and Nasnaran wastes can contribute to the availability of pectin from different botanical sources, allowing for a more diverse and resilient supply chain. Therefore, evaluating the potential of extracting pectin from above fruit wastes in order to minimize the cost of commercial pectin usage in different production processes is highly important.

2. Material and Methods

2.1 Preparation of fruit waste powder

The fresh fruit wastes [Nelli (*Phyllanthus emblica* L.), Starfruit (*Averrhoa carambola* L.), Soursop (*Annona muricata* L.), Avocado (*Persea americana* Mill.), Nasnaran (*Citrus madurensis* L.)] were ground using a laboratory-scale grinder. Ground samples were spread evenly on separated trays and it was dried in oven at 55°C for 24 hours. Then samples were ground to get powders and sieved through 250µm mesh. Commercially available pectin was used as the control.

2.2 Extraction of pectin from prepared fruit waste powder

Distilled water (1:30) was added to the sieved fruit waste powder. 0.5N HCl was added to adjust the pH to 1.5 approximately. Mixture was stirred at 90°C for 4 hours in magnetic hot plate. Then it was allowed to cool for 5min and filtered through muslin cloth. Filtrate was collected and absolute ethanol was added (volume of ethanol was equal to volume of filtrate). Mixture was again stirred for 1 hour in a magnetic hot plate and allowed to precipitate overnight. Coagulated pectin was filtered through muslin cloth and dried in oven at 55°C for 24 hours [9]. Dried pectin was ground using a grinder and was weighed.

2.3 Determination of recovery percentage of extracted pectin

The weight of fruit waste was measured separately. After the extraction of pectin, the weight of pectin was measured separately. The following equation was used to calculate the recovery percentage of extracted pectin.

$$\text{Recovery Percentage} = \frac{\text{Weight of extracted pectin}}{\text{Weight of fruit waste}} \times 100$$

2.4 Characterization of pectin

- **Determination of moisture content of extracted pectin**

One gram of pectin sample was weighed, ground to pass 250-mesh screen (0.0016 inch wire diameter) and placed into a metal dish. The sample was dried in an oven for 5 hours at 100°C, cooled in a desiccator and then it was weighed [9]. Moisture content (dry basis (w/w)) was calculated by using following equation.

$$\text{Moisture Content (\%)} = \frac{\text{Weight of moisture}}{\text{Dry weight of sample}} \times 100$$

- **Determination ash content of extracted pectin**

Two grams of pectin was ground to pass 250-mesh screen (0.0016 inch wire diameter) and placed into crucibles. Then it was ignited in a muffle furnace for 3-4 hours at 600°C [9].

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of pectin}} \times 100$$

- **Determination of equivalent weight of extracted pectin**

Equivalent weight was used to calculate anhydrouronic acid content and degree of esterification. It was determined by titration with NaOH to pH 7.5 using phenol red. Pectin sample (0.5 g) was weighed in to a 250 ml conical flask and moistened with 5ml ethanol. Then 1.0g NaCl was added to the mixture followed by 100ml distilled water and three drops of phenol red indicator. Finally six drops of phenol red was added and titrated against 0.1N NaOH until the color of the solution is pink at the end point [3]. Following

equation was used to calculate the equivalent weight.

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

- **Determination of methoxyl content of extracted pectin**

Methoxyl content was determined using the neutralized solution obtained during the equivalent weight determination, containing 0.5 g of pectic substance, 25 ml of 0.25N NaOH was added to the neutralized solution used in the equivalent weight determination. The mixture was stirred thoroughly and allowed to stand for 30 min at ambient temperature. Then 25 ml of 0.25N HCl (or an amount equivalent to base added) was added and titrated against 0.1N NaOH to the same end point as mentioned above [9]. Following equation was used to calculate the methoxyl content.

$$\text{Methoxyl Content} = \frac{\text{ml of NaOH} \times \text{Normality of NaOH} \times 3.1}{\text{Weight of sample (g)}}$$

- **Determination of anhydrouronic acid content (AUA) of extracted pectin**

Anhydrouronic Acid Content (AUA) is essential to determine the purity and degree of esterification, and to evaluate the physical properties [3]. Following equation was used to calculate the anhydrouronic acid content.

$$\text{AUA (\%)} = \frac{176 \times 0.1Z \times 100}{W \times 1000} + \frac{176 \times 0.1Y \times 100}{W \times 1000}$$

Z = ml of NaOH from equivalent weight determination
Y = ml of NaOH from methoxyl content determination
W = weight of sample
176 = molecular weight of AUA

- **Determination of degree of esterification (DE) of extracted pectin**

The degree of esterification of extracted pectin was calculated from methoxyl and anhydrouronic acid content [3]. Following equation was used to calculate degree of esterification.

$$\text{DE} = \frac{176 \times \text{Methoxyl content (\%)}}{31 \times \text{AUA (\%)}} \times 100$$

- **Determination of color of extracted pectin**

Color of extracted pectin was determined using color reader (CR-10 Plus) by measuring L*, a*, b* values. To get values, tip of the color reader was placed flat against the specimen.

- **Determination of solubility of extracted pectin**

Pectin sample (0.5 g) were moistened with 95% ethanol, dispersed in deionized water. Mixture was stirred in a vortex mix to dissolve. To obtain the hot water soluble fraction, the mixture was heated at 90°C for 15 min. Changes of mixtures were observed visually [10].

2.5 Scanning electron microcopy (SEM) analysis of extracted pectin

Dried pectin powder was fixed onto a SEM specimen stub with a double-sided adhesive tape prior to coating. Specimens were coated using an ion sputter and subsequently photographed using scanning electron microscope (Model: Carl Zeiss) [11].

2.6 Statistical analysis

The experiment was arranged as a Completely Randomized Design (CRD). Data was analyzed using ANOVA in SAS programme with 95% confidence level and mean separation was done using LSD mean separation method.

3. Results and Discussion

3.1 Yield of extracted pectin

Out of the five fruit wastes tested, only three, namely *nasnaran*, soursop and starfruit wastes yielded measurable amount of pectin. The rest of the two fruit wastes, Nelli and Avocado, did not yield significant amounts of pectin through the acid extraction method.

Table 1: Yield of extracted pectin from fruit waste

Type of the fruit waste	Recovery percentage of pectin %
Nasnaran	12.57
Soursop	2.08
Starfruit	2.31
Nelli	ND*
Avocado	ND*

ND* – Significant yield was not detected by using acid extraction method

Nasnaran gave the highest pectin yield while the least value was obtained from soursop (Table 1). Citrus fruits are the most preferred source of pectin extraction due to their high pectin content [12]. That may be the reason for the highest yield of *nasnaran* pectin in this study. Pectin yields might vary with the ripening stage. Ripening will convert the insoluble pectin to soluble pectin [5]. However, over-ripening will cause degradation of pectin and yield less amount of pectin, which might be the reason for less amounts of pectin extracted from Starfruit and Soursop because it was observed that the samples obtained were at the overripe stage.

Over-ripening of fruit, peels might result in a decrease in yield due to the pectin degradation under the action of enzymes, such as polygalacturonase, pectin methyl esterase, or pectate lyase [9].

3.2 Characterization of extracted pectin

- **Moisture and ash content**

The chemical characteristics of the pectin extracted from different fruit wastes are given in Table 2.

Table 2: Moisture and ash content of pectin extracted from fruit waste

	***Nasnaran* pectin**	**Starfruit pectin**	**Soursop pectin**	**Commercial pectin**
Moisture content	$9.49^{c}\pm$ 0.15	$10.53^{b}\pm$ 0.05	$10.20^{b}\pm$ 0.11	$11.61^{a}\pm$ 0.39
Ash content	$5.85^{c}\pm$ 0.29	$9.30^{a}\pm$ 0.17	$8.41^{b}\pm$ 0.10	$3.05^{d}\pm$ 0.27

Note: Values are means ± standard deviations of three replicate measurements. Means with the same letters in the same row are not significantly different (P>0.05)

The lowest (p<0.05) moisture content showed by *Nasnaran* pectin and the control sample showed highest moisture content (Table 2). The maximum limit of moisture content in pectin should not be more than 12% [13]. Thus moisture content of all the pectin samples were in the acceptable range (<12%).

Additionally, it is important for pectin to have a low moisture content in order to ensure safe storage. Higher moisture content can lead to the growth of microorganisms and the production of pectinase enzymes, which can negatively impact the quality of the product [9]. This indicates that all extracted pectin had favorable storage characteristics.The inorganic impurities in pectin were indicated by the ash content and good quality pectin contains lower ash content [3]. Among the evaluated pectin, commercial pectin had the lowest (p<0.05) ash content followed by *nasnaran* pectin. Lower ash content (<10%) is one of the good criteria for gel formation [6]. As fruits ripen, their sugar content and other constituents that can contribute to impurities in pectin tend to increase significantly. [6]. It may be the reason for the lower ash content of pectin extracted from *nasnaran* due to the lower sugar content of fruits. When increasing the strength of the acid used for pectin extraction, the ash content of pectin also increases [14].

- **Equivalent weight, methoxyl content, Anhydrouronic acid content and degree of esterification**

According to Table 3, there was a significant difference (p<0.05) between all treatments and commercial pectin for all the characteristics analyzed. Pectin extracted from Starfruit waste had a higher equivalent weight but was lower than the control and pectin extracted from *Nasnaran* waste had the lowest equivalent weight.

The equivalent weight of pectin can vary significantly depending on the maturity stage of the fruit, particularly when it reaches over-ripeness [6]. Extracted pectin from overripe fruits tends to have a lower equivalent weight compared to pectin extracted from mature fruits, which exhibits the highest equivalent weight.

This difference in equivalent weight can be attributed to various factors associated with fruit ripening. As fruits mature and become overripe, there is an increase in sugar content, which can lead to the breakdown of pectin molecules. This breakdown results in smaller pectin chains and a decrease in the molecular weight of the extracted pectin. Consequently, the equivalent weight, which represents the molecular weight of pectin required to neutralize a given amount of base, is lower in pectin extracted from overripe fruits [6].According to results of this study equivalent weight showed opposite view because the samples obtained from Starfruit and Soursop showed higher equivalent weight results, which were at the overripe stage than *Nasnaran*.

Table 3: Characteristics of pectin extracted from fruit waste

	***Nasnaran* pectin**	**Starfruit pectin**	**Soursop pectin**	**Commercial pectin**
Equivalent weight	$373.83^{c}\pm$ 35.56	$653.22^{b}\pm$ 17.02	$609.78^{b}\pm$ 20.86	1196.81^{a} ± 34.27
Methoxyl content	$7.85^{a}\pm$ 1.22	$3.51^{b}\pm$ 0.36	$4.50^{b}\pm$ 0.32	$1.89^{c}\pm$ 0.09
Anhydrouronic acid content	$91.92^{a}\pm$ 9.63	$46.88^{b}\pm$ 2.65	$54.50^{b}\pm$ 1.82	$25.46^{c}\pm$ 0.79
Degree of esterification	$48.36^{a}\pm$ 3.63	$42.42^{bc}\pm$ 2.11	$46.84^{ab}\pm$ 2.12	$42.18^{c}\pm$ 1.18

Note: Values are means ± standard deviations of three replicate measurement. Means with the same letters in the same row are not significantly different (P>0.05).

Methoxyl content is an important factor in determining the gel formation capacity [15]. According to the Table 3, pectin extracted from *nasnaran* showed the highest (p<0.05) methoxyl content followed by pectin extracted from star fruit, soursop and control. In this study methoxyl contents of extracted pectin were lower than the pectin extracted from pumello peel, lime peel and mangosteen rind and mango peel [3,16]. However, it was higher than pectin extracted from dragon fruit peel [17]. Depending on the source of the raw

material and on the mode of extraction, methoxyl content of extracted pectin from plant resources generally varies from 0.2% - 12%. All the extracted pectin from the selected fruit wastes were in this range of methoxyl content. Low methoxyl pectin is an indicator of good quality pectin for food processing and it is preferable for low sugar diet foods because it can form gels with lower sugar amount or even without sugar in divalent cations [4]. Due to ripening, sugars content of fruits are increased with the decreasing of methoxyl content [6]. That could be the reason for lower methoxyl content in the pectin extracted from Starfruit waste and Soursop peel.

Anhydrouronic Acid Content (AUA) is essential to determine the purity, degree of esterification and to evaluate physical properties and it should not be less than 65%. In this study the highest ($p<0.05$) AUA content was found in the pectin extracted from *nasnaran* and lowest ($p<0.05$) AUA content was found in the commercial pectin. Pectin extracted from fruit wastes often undergoes simpler and more gentle extraction processes. These extraction methods, such as enzymatic or acid extraction, can minimize the introduction of impurities into the final pectin product. Furthermore, commercial pectin formulations may include additives and stabilizers to enhance their functionality and shelf life. These additional ingredients as well as residual pesticides, waxes, and other unwanted substances that are difficult to completely remove during the manufacturing process can introduce impurities into the pectin.

Pectin extracted from Starfruit waste and Soursop peel had AUA less than 65%. High AUA indicate the higher purity and impurities may contain due to presence of proteins, starch and sugars [9] in the precipitated pectin because alcohol precipitation alone would not be able to eliminate that impurities [4].

Pectin can be categorized into two categories depending on degree of esterification (DE) such as high methoxyl pectin (DE > 50%) and low methoxyl pectin (DE < 50%) [3]. According to results of this study, the degree of esterification was ranged from 42.42 ± 2.11 to 48.36 ± 3.63 and all pectin types were rated as low methoxyl pectin.

- **Color of extracted pectin**

Table 4 presents the color values of the extracted pectin. The L*, a*, and b* values in the table represent the lightness, redness, and yellowness, respectively. It was observed that the pectin extracted from starfruit exhibited significantly higher L*, a*, and b* values ($p<0.05$) compared to the pectin extracted from nasnaran and soursop. The higher values indicate a lighter and more vibrant color in the starfruit pectin.

The darker color of pectin can be attributed to the presence of polyphenols or other water-soluble pigments that become trapped within the pectin during the precipitation process [18]. These pigments contribute to the darker color of the pectin extracted from starfruit waste compared to the pectin derived from nasnaran and soursop.

Table 4: Colour of pectin extracted from fruit waste

Color values	***Nasnaran* pectin**	**Starfruit pectin**	**Soursop pectin**
L*	$36.53^{b} \pm 0.15$	$40.60^{a} \pm 0.56$	$35.73^{b} \pm 0.45$
a*	$2.13^{b} \pm 0.21$	$5.13^{a} \pm 0.49$	$1.20^{c} \pm 0.17$
b*	$3.17^{b} \pm 0.31$	$6.67^{a} \pm 0.55$	$2.40^{b} \pm 0.46$

Note: Values are means ± standard deviations of three replicate measurements. Means with the same letters in the same row are not significantly different ($P>0.05$)

- **Solubility of pectin extracted from fruit waste**

Table 5: Solubility of pectin in cold and hot water

Fruit type	**Solubility in cold water**	**Solubility in hot water**
Nasnaran pectin	Partly soluble suspension	Soluble suspension
Starfruit pectin	Partly soluble turbidity	Partly soluble high turbidity
Soursop pectin	Partly soluble and precipitated	Soluble and precipitated
Commercial pectin	Soluble suspension	Soluble suspension

Nasnaran pectin shows partial solubility in cold water but becomes fully soluble in hot water. Starfruit pectin exhibits partial solubility with turbidity in both cold and hot water. Soursop pectin demonstrates partial solubility with precipitation in cold water and solubility with precipitation in hot water. On the other hand, the commercial pectin used for comparison shows complete solubility in both cold and hot water. These solubility differences can be attributed to variations in the composition and structure of the pectin extracted from different fruit sources.According to the results, a decrease in the esterified carboxylic group (DE) reduced the solubility of extracted pectin. High ash content and the drying process also may affect to the reduction of pectin solubility [10].

- **Micrograph of extracted pectin**

Figure 1 displays the microstructure morphology of pectin extracted from Nasnaran, Soursop, and Starfruit (shown as Figure 1a, 1b, and 1c, respectively). Upon observation, it was found that Nasnaran pectin exhibited a rough porous surface with the presence of some fibers. Soursop pectin, on the other hand, displayed a rough flaky surface, while Starfruit pectin showcased a rough flaky corrugated surface.

The variations in microstructure morphology can impact the solubility and functionality of pectin. Pectin particles with a more porous structure tend to have enhanced solubility compared to those with rigid structures and lower porosity. This is because particles with greater porosity offer more surface area for contact with the solvent, facilitating the dissolution process. As a result, the solubility of Nasnaran pectin in both cold and hot water may be improved due to its rough porous surface and the presence of fibers [4].

Moreover, the presence of a rough flaky surface in Soursop pectin and a rough flaky corrugated surface in Starfruit pectin suggests potential differences in their structural properties. These variations in surface morphology can influence the interaction between pectin particles and the solvent, affecting the overall solubility and viscosity of the pectin solution.

4. Conclusion

In this study, the highest pectin yield was obtained from *nasnaran* waste by conventional extraction method. Lower moisture and ash content, higher anhydrouronic acid content, and solubility showed by pectin extracted from *Nasnaran* fruit waste. Among the evaluated fruits, the equivalent weight of *nasnaran* was significantly lowerand the methoxyl content was significantly higher compared to other fruits. The degree of esterification of all pectin types was denoted as low methoxyl pectin. Thus it can be concluded that the pectin extracted from *nasnaran* waste could be potential alternative sources for high quality pectin for the use in food industry.

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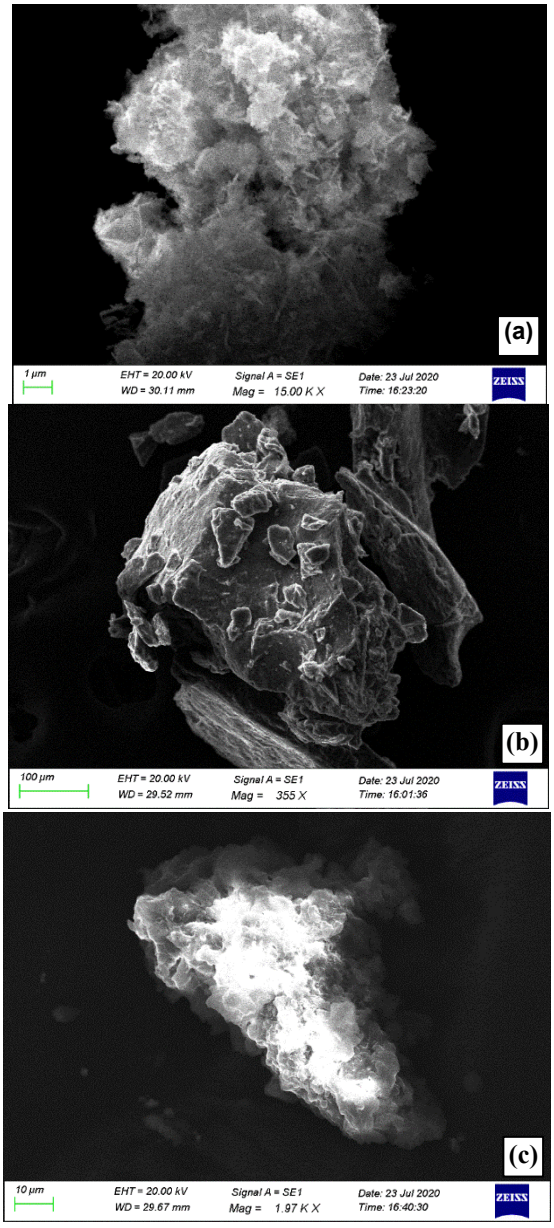

Figure 1: Scanning electron microscopic images (a) Nasnaran pectin (b) Soursop pectin (c) Starfruit pectin

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