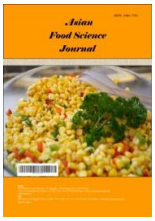

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Isolation and Characterization of Pectin from Pumpkin (*Cucurbita maxima*) Waste and Its Food Application

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Pectin was extracted using the method of acid hydrolysis followed by ethanol precipitation at various reaction conditions. Effect of blanching prior to acid hydrolysis, acid types (hydrochloric and citric) and extraction time and temperature combinations were tested to maximize the yield of pectin and they were characterized by assessing physiochemical and functional properties. The feasibility of utilizing extracted pectin in food applications was evaluated. The yield of pectin ranged from 7.30 ± 0.07- 11.04 ±0.01% on a dry weight basis. The results suggested that the highest pectin content is in the fresh pumpkin peel extracted using 0.1 N hydrochloric acid at 80±5℃, 1.25 pH for 1 hour. Water holding capacity, oil holding capacity and emulsifying activity index of pumpkin pectin were 2.5 g/g of pectin (SD 0.46), 1.76 g/g of pectin (SD 0.10) and 0.29 (SD 0.01) respectively. In addition, the degree of esterification, equivalent weight, methoxy content and galacturonic acid content of extracted pectin were 67.64% (SD 0.89%), 978.35 (SD 69.88), 6.55% (SD 0.37%) and 66.46% (SD 1.19%) respectively. Sensory analysis revealed that there is no significant difference in spreadability, surface texture and chewiness of two types of jam prepared using pumpkin and commercial pectin. In conclusion, pumpkin pectin can be categorized as high methoxy pectin.

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Keywords: Acid hydrolysis assisted extraction; galacturonic acid; high methoxy pectin; pumpkin waste.

1. INTRODUCTION

Pumpkin (*Cucurbita maxima*) is a short-aged crop widely grown in intermediate and dry zone of Sri Lanka [1]. It is frequently consumed as a main course or a side dish and used as an ingredient in pies, soups, stews, and bakery preparations [2,3]. In Sri Lanka, annual production of pumpkin is about 12063 Mt per year [4]. It has found that there is a higher postharvest loss of pumpkin (10.23%) in the seasonal period at Dambulla Economic Centre which is the focal point of fruit and vegetable transaction in Sri Lanka [5].

There is a great potential to utilize pumpkin waste to extract value-added ingredients such as pectin. Pumpkin is known to be a low-cost source of pectin [6] and pectin content is ranging from 8-22% on a dry weight basis [7]. However, the availability of pectin can be changed due to climatic and soil conditions [8].

Pectin is a complex polysaccharide made out of D-galacturonic acid, L-rhamnose, L-arabinose, and D-galactose [9]. Pectin is widely used in the food industry as a thickener, emulsifier, texturizer, stabilizer and gelling agent in jams and jellies. It is further used for fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products [10]. In addition, pectin has been shown to lower blood cholesterol levels especially low-density lipoprotein cholesterol fractions and reduces the risk for coronary heart diseases [11].

Pectin has a great structural diversity according to the type of waste material such as mango peel, banana peel, buttercup, sugar beet pulp and squash fruit which is used for the extraction [8]. It can result in different physicochemical properties of pectin which can be used in a wide range of applications [9]. It is important to investigate new sources of pectin in order to meet domestic and regional demands [12]. There are limited research studies that have been focused on the extraction of pectin from Pumpkin [13].

The present study is focused to extract and characterize pectin from pumpkin waste as an alternative source to apple and citrus pectin. In addition, the study investigated the effect of different treatments on the yield of extracted pectin.

2. MATERIALS AND METHODOLOGY

2.1 Raw Material Collection

Waste materials of pumpkin were obtained from the local market, Pannala, Sri Lanka.

2.2 Pretreatment of Waste Materials of Pumpkin

Spoiled and rotten parts of pumpkin were removed at the initial stage and the rest were washed well and cut into small pieces. Those pumpkin pieces were separated into four samples and subjected to different treatments as blanched fresh, un-blanched fresh, blanched dried and un-blanched dried in order to evaluate the effect of blanching and drying on the yield of pectin.

Blanched fresh- Samples were blanched using hot water at 60±2℃ for 5 minutes and cooled to room temperature; Blanched dried- Samples were blanched using hot water at 60±2℃ for 5 minutes and cooled to room temperature then dried at 50±2℃ for overnight; Un-blanched dried-Fresh samples were dried at 50±2℃ overnight. Control sample was not subjected to any treatment. All prepared samples were packed in polyethylene bags and kept in freezer at -18±1℃ until use for pectin extraction. Pretreated samples were ground into fine particles and as starting material as per the design of extraction.

2.3 Extraction of Pectin

Pre-treated ground samples (100 g) were added with 0.1N hydrochloric acid (200 mL) and pH was adjusted to 1.25. Samples were heated to 80±2℃ in a water bath for 60 minutes under continuous stirring followed by cooling to 20±1℃ and centrifugation at 6000rpm for 10 minutes. The supernatant was filtered through a muslin cloth. Then 400 mL of 95% ethanol was added to the supernatant and kept for 16 hours followed by centrifugation in order to recover pectin. 70% ethanol was added to the separated precipitate for purification of extracted wet pectin and it was dried at 40±1℃ for 48 hours in a hot air oven and powdering was done using a grinder

(HL1643/04) to obtain pectin powder (Modified method of Liew et al. [14].

2.4 Effect of Acid Type, Extraction Temperature and Extraction Time on Yield of Pectin

Pectin was extracted using 0.1N hydrochloric acid and citric acid at four different temperatures: 60, 70, 80 and 90℃. The extraction time period was varied as 30, 60, 90 and 120 minutes.

2.5 Percentage of Pectin Yield

Pectin yield was calculated using the following equation.

> The yield of pectin = Weight of dried pectin × 100% / weight of the sample taken for extraction

2.6 Physicochemical and Functional Properties of Pectin

2.6.1 Determination of equivalent weight

Pectin (0.5 g) was weighed accurately using an analytical balance. The weighed sample was transferred into a 250 ml conical flask and 5 ml of 70% ethanol was added. Sodium chloride (1 g) and 100 ml of distilled water were added to the conical flask. Few drops of phenol red were added and titrated with 0.1 N sodium hydroxide. Equilibrium point was indicated by pink colour [10]. Equivalent weight was calculated using the following equation

> Equivalent weight = Weight of alkali ×1000 / Normality of alkali × mL of alkali

2.6.2 Degree of esterification

The degree of esterification of pectin was determined by titration [14]. Dried pectin (0.2 g) sample was weighed using analytical balance. 0.1 mL of ethanol was added to the sample to moisten pectin. The sample was dissolved in 20 ml distilled water. It was placed in an automatic shaking water bath at 45°C until the pectin dissolved completely. Three drops of phenolphthalein were added into the sample. The sample was titrated with 0.1 N sodium hydroxide solution. The free carboxyl group was calculated from the volume of 0.1 N sodium hydroxide solution spent for initial titration. 0.1 N sodium hydroxide (10 mL) was added to neutralize polygalacturonic acid of pectin. The sample was plugged with a stopper and shaken vigorously, then allowed to stand at room temperature for 2 hours for de-esterify pectin. 0.1 N hydrochloric acid (10 mL) was added again to neutralized sodium hydroxide and the sample was shaken until its pink colour disappeared. Three drops of phenolphthalein were added into the sample and the sample was further titrated with 0.1 N sodium hydroxide. The volume of titration was recorded when the colour of the indicator turned in to pink. The number of the esterified carboxyl group was calculated from the volume of 0.1 N sodium hydroxide solution spent for final titration.

> Degree of esterification = (final titration volume) / (initial titration volume + final titration volume) × 100%

2.6.3 Methoxy content

The neutral solution of equivalent weight determination was collected. It was treated with 25 mL of 0.25N sodium hydroxide. The solution was stirred vigorously and kept for 30 minutes at room temperature. After 30 minutes, 25 mL of 0.25N hydrochloric acid was added and the solution was titrated against 0.1N sodium hydroxide until the endpoint [15].

> Methoxy content = (mL of alkali x Normality of alkali x 3.1 / Weight of sample)

2.6.4 Galacturonic acid content

When the Equivalent Weight and methoxyl content of pectin is known, its Urological Association (AUA) was calculated as follows [15].

> AUA = [176 (M.Eq. of alkali free acid+ M.Eq. alkali for saponification+ M.Eq. for ash) / Weight of sample]

2.6.5 Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC were determined according to the method of Gannasin et al. [16] with some modifications.

Distilled water or coconut oil (25 mL) was added to 250 mg of dry pectin sample, stirred and left at room temperature for 1 h. After centrifugation, the residue was weighed. The WHC was expressed as g of water held per g of sample, while the OHC was expressed as g of oil held per g of sample [16].

2.6.6 Emulsifying activity index (EAI)

Emulsifying Acidity Index was determined according to the method described by Soares et al. (2009) with slight modifications. Pectin (1 g) was dissolved in 50 mL of distilled water, pH was adjusted to 6.2. Solution was stirred for 60 minutes using magnetic stirrer. 40 mL of distilled water was added and volume as adjusted to 100 mL. Oil/water emulsion was prepared by adding 15 mL of the solution into 5 mL of coconut oil. The emulsion was homogenized for 90 s using a vortex mixer. 3 mL of the aliquot was removed and diluted with 200 mL of 0.1% sodium dodecyl sulphate. Absorbance was measured at 500nm. EAI was expressed directly as the absorbance at 500 nm.

2.7 Sensory Analysis of Pectin

Sensory characteristics of commercial pectin and pectin extracted from pumpkin waste were compared using a ranking test. Ranking test was conducted in order to determine the difference of textural qualities of two jam samples related to spreadability, chewiness and surface texture.

Fresh pineapple was cut into small pieces and ground in order to obtain the pulp. Pineapple pulp (45 g) was heated in saucepan to 105℃. Then citric acid (0.53 g) and sugar (55 g) were added to the mixture. It was stirred until it reaches to a semi-solid texture and 65% of total soluble solids. The semi-solid mixture was filled into pre-sterilized lug jars while they are hot. Then lug jars were cooled in refrigerator.

Sensory analysis was conducted using 30 untrained panelists and cream cracker biscuit was used as the carrier food for jam.

3. RESULTS AND DISCUSSION

3.1 Yield of Pectin

Percentage yield of pectin varied from 1.0 to 3.0% according to the different parts of pumpkin used for the pectin extraction and pretreatment (Table 1). Pectin yield of peel was higher than the core and whole fruit. Core has the lowest yield. Pectin was found in cell walls of plants as an integral structural component of cells which helps to give them rigidity. Hence the hard parts of fruits contain more pectin than the soft parts and it confirmed by the results obtained from the study as peel reported the highest yield compared to internal parts [17].

3.2 Effect of Pretreatments

According to the results in Table 1, highest yield of pectin was observed for the un-blanched fresh pumpkin. The yield of pectin obtained from blanched dried powder was at an intermediate level. Blanched dried powder form is commonly used for industrial pectin production, which is due to long storage time and reduction in transport costs and labor costs (Delavault et al., 2010). Yield obtained for unblanched dried sample was the lowest due to the degradation of pectin by the action of pectinolytic enzymes. [18] during drying which takes the time that causes quality and the yield of pectin have been reduced. Thus, blanching followed by drying are important steps before subject the samples for pectin extraction, however, according to the results, the yield of pectin can be optimized if the fresh raw material is used for the extraction.

3.3 Effect of Acid Type on the Yield of Pectin

The effect of acid type on the yield of pectin is illustrated in Fig. 1. There was a significant effect ($p < 0.05$) of acid type on yield of pectin. In contrast, Schemin et al. (2016) reported that no significant effect of type of acid used for the extraction on the yield of pectin from apple. Our study has shown that less influence is given by citric acid in enhancing the yield of pectin as it provides the lowest yield that compared the HCl acid within the given time of 60 min (Fig. 2). However, it has been reported that citric acid is known to be the least pectin degrading (depolymerizing and de esterifying) extracting agent [19].

The highest yield was obtained for the hydrochloric acid from core, peel and the whole fruit (Fig. 2). Presence of high concentration of hydrogen ions stimulated the hydrolysis of pectin from proto pectin. The higher ionic strength of hydrochloric acid has an improved capability to precipitate pectin due to their higher affinity for cations such as Ca^{2+} which stabilizes the pectin molecule [20]. However, Yapo, [19] reported that acid type strongly influenced the macromolecular and gelling properties of isolated pectin.

3.4 Effect of Extraction Time on the Yield of Pectin

The effect of the different extraction periods on the yield of pectin is illustrated in Fig. 2.

There is a significant effect ($p < 0.05$) of extraction time on the yield of pectin.

The highest yield was obtained at 60 minutes of extraction using HCl, while the lowest at 30 minutes for the same acid. An extraction time of 90 minutes showed an intermediate yield. The results showed that the yield of pectin increased with increase in extraction time up to (what time? but after the optimized level yield has been decreased with the extraction time. Interestingly when using the citric acid (weak acid) it has taken more time but provides optimized yield and the relationship with extraction time and yield is observed as the same. Excess extraction time may lead to decomposition of pectin structure. Glycosidic bonds and ester bonds are broken due to higher extraction time. Similar results were reported for pectin extracted using orange peels [21].

3.5 Effect of Extraction Temperature on Yield of Pectin

The effect of the different extraction periods on the yield of pectin is illustrated in Fig.3.

There was a significant effect ($p < 0.05$) of temperature on the yield of pectin. Yield at 60℃ was increased by 3 folds at extraction of 80℃ within the similar time duration. The heated acid helped to solubilise pectin and other pectic components held in the cell wall (proto pectin), thereby increased the yield of pectin. Low temperature may be insufficient to permit the hydrolysis of protopectin by acids, thus obtaining a lower yield of pectin [19].

The results revelled that time-temperature combination is needed to highly consider to obtain maximum yield of pectin and this will be further effect by the acid type that used for the pectin extraction.

Table 1. Percentage yield of pectin extracted from pumpkin waste with different pretreatments

Pretreatment	% Yield of pectin from whole fruit	% yield of pectin from core	% yield of pectin from peel
Blanched fresh	2.07 ± 0.02[a]	2.03 ± 0.06[a]	2.54±0.06[a]
Un blanched fresh	2.28 ± 0.02[b]	2.52± 0.21[b]	2.91±0.06[b]
Blanched dried	1.88 ±0.24[c]	1.64± 0.04[c]	2.54±0.32[b]
Un blanched dried	1.24 ±0.06[d]	0.95±0.06[d]	2.42±0.09[b]

Data given as mean ± standard deviation from triplicate analysis

Values with different superscript in the same column are significantly different ($p< 0.05$) by LSD mean separation

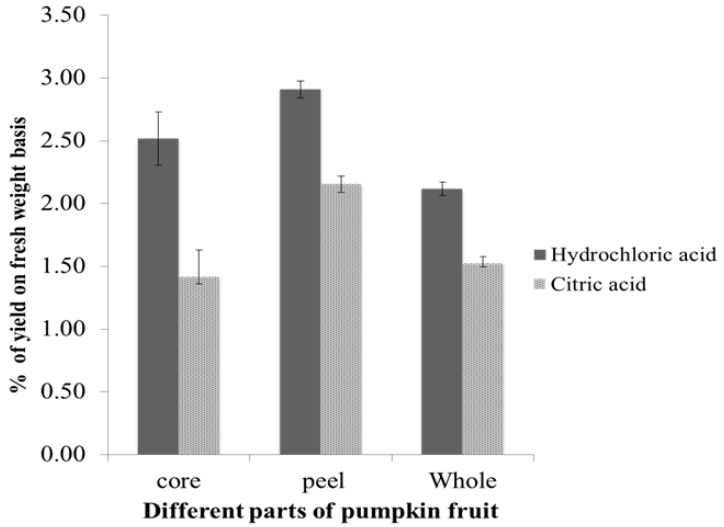

Fig. 1. Effect of acid type on yield of pectin

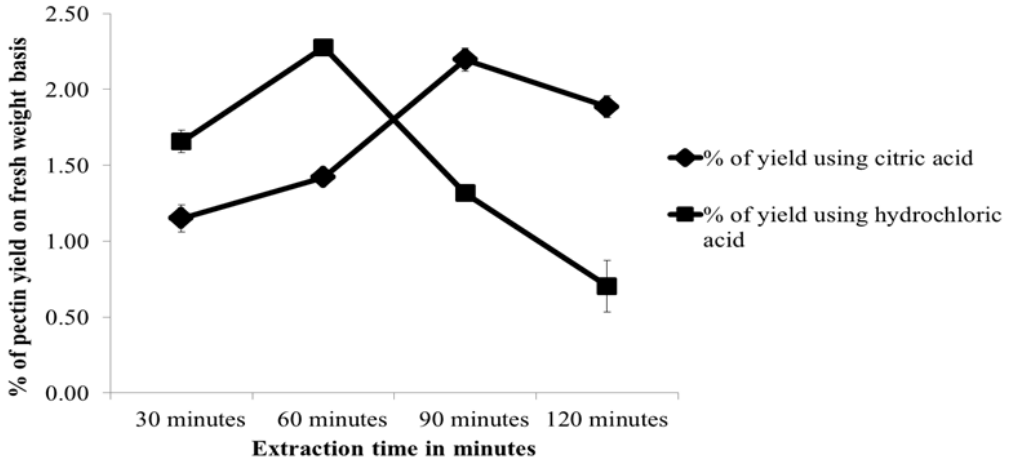

Fig. 2. Effect of extraction time on yield of pectin

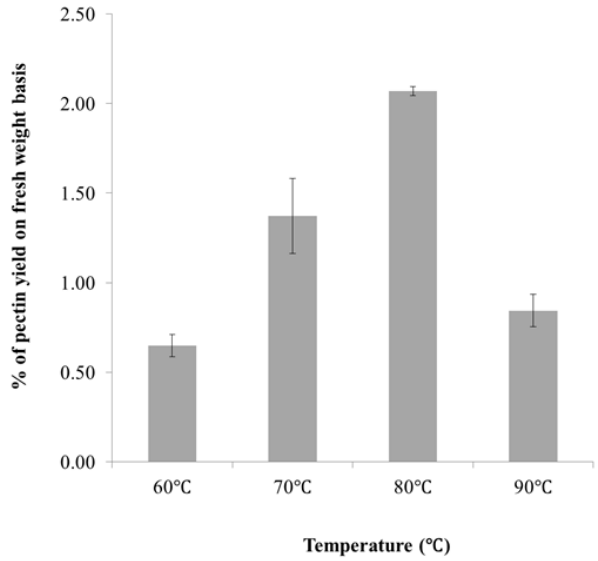

Fig. 3. Effect of extraction temperature on yield of pectin

3.6 Physiochemical and Functional properties of Pectin

3.6.1 Degree of esterification (DE)

The methyl esters and amide groups of pectin play a major role for the functionality of pectin. The degree of esterification provides information on the kinetic behaviour during gel formation which can be differed for high methoxy and low methoxy pectins. Our results showed that the degree of esterification of pumpkin pectin was 67.64 and thus pumpkin pectin can be categorized as high methoxy pectin because the degree of esterification is higher than 50. Therefore, extracted pumpkin pectins do not require calcium ions for gel formation. Similar DE values for pumpkin pectin has been reported by Yoo et al. [13]. Notably, pectin extracted from pumpkin has a great potential to utilize in jams and jellies as a gelling agent and a thickener.

3.6.2 Equivalent weight

Equivalent weight of extracted pectin was significantly higher ($P<0.05$) than the values of commercial pectin. This may be due to differences in methodology and acids used for the extraction. Equivalent weight can affect the solubility and gelation properties. The increased

or decreased of the equivalent weight might be also dependent upon the amount of free acid.

But lower values were reported for the pectin extracted from passion fruit rind [22]. Furthermore, lower values also reported in pectin extracted from guava peel [15].

3.6.3 Galacturonic acid content

AUA of pumpkin pectin was 66.67%, which was higher than the pectin extracted from mango peel (56.67%) but lower than the citrus pectin. Similar values were reported for pectin extracted from jack fruit rind [23]. In addition, higher AUA contents were reported for palmyra palm pectin [8]. Lower AUA contents were reported in pectin extracted from cinnamon apple pomace [24].

3.6.4 Methoxy content

Methoxy content is an important factor in controlling the setting time of pectin, their combining power with metallic ions and the ability of pectin to form gels [15]. Methoxy content of extracted pectin is 6.55%. It was lower than the commercial citrus pectin. Reduced methoxy content may be due to the slight differences in pectin structure or difference in the extraction conditions of pectin. Methoxy content of pectin extracted from mango, jackfruit rind and banana pectin was reported as 7%, which is closer to the value of pumpkin pectin [23].

3.6.5 Water holding capacity (WHC) and oil holding capacity (OHC)

WHC of extracted pumpkin pectin was slightly lower than the commercial pectin and apple pectin (Gannaasin et al., 2012), but at an acceptable level which can be utilized to reduce syneresis and to modify texture of foods. OHC of extracted pectin is higher than the commercial pectin. Similar values were reported in for apple pectin, bovine gelatin and agar (Gannasin et al., 2012) Higher OHC of pectin is important in the food industry; as an emulsifier in acidified dairy drinks.

3.6.6 Emulsifying Activity Index (EAI)

EAI of extracted pectin (0.27) and commercial pectin (0.31) did show any difference in the present study, however, EAI of commercial pectin was reported as 0.27 in some studies (Soares et al., 2009). EAI is important for emulsifying properties of pectin and to use in acidified dairy drinks and yoghurts. Further, EAI can depend upon the extraction conditions and type of raw material used for the extraction. Physiochemical and functional properties of pectin are given in Table 2.

3.6.7 Sensory analysis of pectin

Two jam samples prepared from commercial pectin and extracted pumpkin pectin were not significantly ($P > 0.05$) different in terms of surface texture, chewiness and spread-ability.

Table 2. Physiochemical and functional properties of pectin

Physiochemical/functional property	Commercial pectin	Pumpkin pectin
Degree of esterification	77.50±0.92	67.64±0.89
Equivalent weight	866.63±15.84	978.35±69.88
Methoxy content	8.133± 0.69	6.55±0.37
Water holding capacity	3.50±0.32	2.51±0.46
Oil holding capacity	1.52±0.25	1.76±0.1
Emulsifying activity index (EAI)	0.31±0.01	0.29 ±0.01

Data given as mean ± standard deviation from triplicate analysis

Table 3. Sensory analysis of pectin

Textural property	Mean score of commercial pectin jam	Mean score of extracted pectin jam
Spreadability	4.4±0.2	4.1±0.1
Chewiness	4.2±0.1	4.2±0.1
Surface Texture	4.2±0.1	4.5±0.1

Data given as mean ± standard deviation

4. CONCLUSION

The results revealed that time temperature combination should be highly considered to obtain the maximum yield of pectin and this will be further effect by the acid type that used for the pectin extraction. Pectin extracted from pumpkin can be categorized as high methoxy pectin and higher pectin yield indicated that pumpkin is an alternative raw material for pectin extraction.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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