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Isolation of Pectin from *Nephrolepis Biserrata* Leaves at Different Extraction Time

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Graphical abstract

pH of Pectin	Temperature (°C)	Extraction Time (min)	Yield (%)
1.5	80	60	2.0
		90	5.15
		120	7.9

Abstract

Based on a previous test (fractionation) on *Nephrolepis biserrata*, lignocellulose contains 34.27% of pectin and oligosacharides, 1.78% of hemicellulose, 20.66% of cellulose and 43.29% of lignin. The optimum time for extraction of pectin from *Nephrolepis biserrata* leaves was investigated. Extraction time of 60, 90, 120 minutes at pH 1.5 and temperature of 80°C were found to significantly affect the extraction of pectin from *Nephrolepis biserrata* leaves. Maximum pectin yield is 15.8%, which was obtained in sulphuric acid solution of pH 1.5 at 80°C for 120 minutes.

Keywords: Nephrolepis biserrata leaves; fractionation, Lignocellulose content; extraction time, pectin

Abstrak

Berdasarkan ujian (pemeringkatan) terhadap *Nephrolepis biserrata* yang sebelumnya, lignoselulosa mengandungi 34.27% pektin dan oligosaccharides, 1.78% hemiselulosa, 20.66% selulosa dan 43.29% lignin. Masa yang optimum untuk mengekstrak pektin daripada daun *Nephrolepis biserrata* telah disiasat. Pengekstrakan masa dengan 60, 90, 120 minit pada pH 1.5 dan suhu 80°C didapati amat menjejaskan pengekstrakan pektin daripada daun *Nephrolepis biserrata*. Jumlah penghasilan pektin yang didapai daripada larutan asid sulfurik dengan pH 1.5 pada 80°C selama 120 minit adalah maksimum, iaitu 15.8%.

Kata kunci: daun *Nephrolepis biserrata*; pemeringkatan, kandungan lignoselulosa; masa pengekstrakan; pektin

1.0 INTRODUCTION

Pectin is one of the major components of primary cell wall and it is generally thought to account for about one third of all primary cell wall macromolecules. The middle lamella and the primary cell wall of higher plants contain complex hetero polysaccharide called pectin. These carbohydrate polymers support the cohesion of other cell wall polysaccharides and protein. Pectin is composed mainly of galacturonic acid residues[1].

Pectins are group of polysaccharides consisting mostly of D-galacturonic acid and galacturonic acid methyl ester residues interspersed with a few (1→2)-linked L-rhamnose residues, which are linked to neutral sugar side-chains, such as L- arabinose, D-galactose, D-xylose, D-mannose, and D-glucose [2]. They are polysacharides that act as a cellular binder in the peel of many different fruits and vegetables [3]. Pectins are complex carbohydrate molecules and extensively utilized in food processing especially for the conversion of low grade fruits into good quality products like jam, jelly, marmalade and candies [4].

The yield and quality of pectin depends mostly upon the source as well as the method employed for extraction of pectin. Clearly, relationships between pectin's fine structure and functional properties do exist [5]. Pectin is usually extracted by suspending fruit and vegetable waste in different mineral acid, salt solution, at certain pH and temperature [4]. A research on the effects of temperature and extraction time on pectin yield has been conducted. The highest yield of pectin (16:32%) was obtained at temperature of 95 °C for 80 minutes [6].

The preliminary study in optimization of pectin extraction from cocoa husks under various conditions (pH and extraction time), had constituted the highest yield of pectin is 8.0 % at pH 2.5 and extraction time of 1 hour [7]. Meanwhile, pectin extraction on dried apple pomace and optimization of the effects of processing parameters of extraction on the yield of pectin has been done. The optimum conditions of pectin extraction were found to be at extraction time of 20.8 min, pH of 1.01, giving pectin yield of 0.315 g [8].

The pectins extracted from sugar beet by acid (HCl or HNO_3) at varied pH (1–3), temperature (75–90°C) and time (30–90 min) exhibited a galacturonic acid content and an extraction time varying from 295 to 528 mg/g (dry weight) and 34% to 94%, respectively [5]. Several other researches were also widely carried out in extracting pectin from plants or waste of fruits and vegetables such as apples, oranges, mangos, bananas, tomatoes, potatoes and carrots.

At the moment, researches on extraction of pectin from lignocellulose other than fruits and vegetables are rarely performed, and research on the extraction of pectin from *Nephrolepis biserrata* has not been studied. Therefore it is necessary to study *Nephrolepis biserrata.*. *Nephrolepis biserrata* (Sw) is very easy to find in Malaysia and in other agrarian countries. *Nephrolepis biserrata* has potential as forage in countries with rivers, stream, brooks, oasis, swamps and dams [9]. *Nephrolepis biserrata* is rich in crude protein content, which is the highest in its leaf [10]. Therefore it can be used as an alternative to produce pectin, as it is cheap and easily obtainable.

■2.0 EXPERIMENTAL

2.1 Material and Methods

2.1.1 Sample

Fresh *Nephrolepis biserrata* leaves were collected within the vicinity of Universiti Teknologi Malaysia.

2.1.2 Fractionation of Lignocellulose

1 g of *Nephrolepis biserrata* leaves was dried then refluxed for 2 h with 300 mL of H_2O at 100°C. Dry weight was recorded for pectin and oligosacharides. Dried residue was refluxed for 2 h with 300 mL of 0.5 M H_2SO_4 at 100°C to obtain hemicellulose. The dried residue treated with 20 mL of 72% (v/v) 0.5 M H_2SO_4 at room temperature for 4 h, then diluted with 300 mL of 0.5 M H_2SO_4, and refluxed at 100°C for 4 h to obtain cellulose and lignin.[11]

2.1.3 Pectin Extraction

A total 5 g of *Nephrolepis biserrata* leaves were dried in an oven at 55°C until a constant weight is reached. Dry weight was recorded for pectin yield determination. The dried *Nephrolepis sp* leaves was blended and kept at a substrate to water ratio of 1:40. The desired pH of the mixture was adjusted with 0.5 N Sulphuric acid for a pH of 1.5, then incubated at a temperature of 80°C for different extraction times (60-90-120 minutes) with frequent stirring. After incubation, the contents were filtered through filter paper and pectin from the filtrate was precipitated with 95% ethanol, and then washed twice with ethanol. The obtained pectin was dried in an oven at 40°C until a constant weight is reached and ground finely to analyse its chemical quality characteristic. Yield was calculated by the weight of dried pectin (g) per 100 g of dried *Nephrolepis biserrata* leaves. The extraction scheme for pectin is shown in Figure 1.

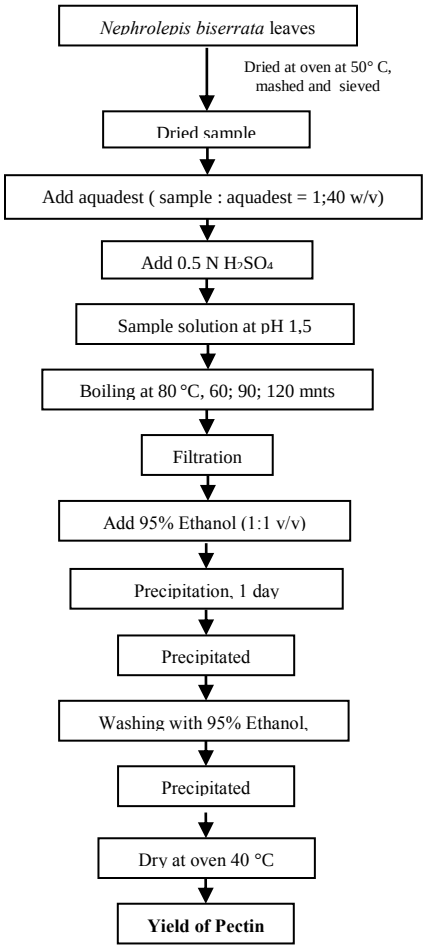

Figure 1 Scheme for the extraction of pectin from *Nephrolepis biserrata* leaves

3.0 RESULTS AND DISCUSSION

Based on a previous test (fractionation) on *Nephrolepis biserrata* leaves, lignocellulose content of pectin, oligosacharides, emicellulose, cellulose, and lignin were found by using soluble hot water and 0.5 M sulphuric acid at 100 °C for 4 h and is shown in Table 1.

The data presented in Table 2 shows that pH, incubation temperature and time distinctly affected the extraction of pectin. Extraction of pectin at 60, 90 and 120 minutes yielded pectin of 2.0%, 5.15% and 7.9 %, respectively (Table 2), at pH 1.5 of the solution and temperature of 80 °C. It is thus apparent from the results that the maximum yield of pectin was obtained by soaking the leaves in a solution with pH 1.5, at the temperature of 80 °C and extraction time of 120 minutes.

Table 1 Lignocellulose content fractionated from *Nephrolepisbiserrata* leave

Type of Lignocellulose content	Composition of Lignocellulose (%)
Pectin, Oligosacharides	34.27
Hemicellulose	1.78
Cellulose	20.66
Lignin	43.29

Extractability of pectin was also affected by the extraction time that ranges from 60 to 120 minutes. The lowest yield (2%) was obtained from sample incubated in solution with pH 1.5 and heat treated for 60 minutes. On the other hand, optimum yield (7.9 %) was obtained from sample adjusted to pH 1.5, heat treated for 120 minutes.

Generally, the duration for sample heat treatment has an influence towards pectin yield. A previous finding on pectin extraction carried out at low pH value, investigating on the possibility to improve the yield, obtained 8.0% yield at pH 1.5 for 2.5 h [1].

Table 2 Pectin yield extracted from *Nephrolepis biserrata l*eaves

pH of Pectin	Temperature (ºC)	Extraction Time (min)	Yield (%)
1.5	80	60	2.0
		90	5.15
		120	7.9

4.0 CONCLUSION

Fractionation method can be use to analyze the structure of plant cell wall of polysacharides. The results of pectin, oligosacharides, hemicellulose, cellulose and lignin are obtained. It is necessary to study and research on the extraction of pectin from *Nephrolepis biserrata*. Hot acid extraction, usually utilized for commercial pectin production, is highly suitable for the recovery of compounds from fruit and vegetable waste. The best result, in terms of extraction yield, is obtained in sulphuric acid solution, with pH 1.5, temperature of 80 °C (7.9%) and incubation time of 120 minutes. When the extraction time was 60 minutes, the yield was lower (2%). Ethanol 95% can be successfully used as a precipitating agent for maximum recovery of pectin from the extracted filtrate.

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