

OPTIMAL CONDITIONS FOR EXTRACTING HIGH-QUALITY PECTIN FROM WATER HYACINTH (*Eichhornia crassipes*) FOR LESS SOLUBLE HYDROGEL PRODUCTION

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ABSTRACT. The research aimed to extract high-quality pectin from water hyacinth for less soluble hydrogel production. The product adds value to water hyacinth waste and helps solve environmental problems. The high degree of esterification pectin can be prepared as a hydrogel, which can be used in various pollution treatment applications. The quality of pectin depended on raw materials and extraction conditions. The optimum condition was initially predicted using the response surface method (RSM). Three extraction variables were studied, including pH 1.0-4.0, temperature 50-110°C, and extraction time 30-240 min. A total of seventeen runs including five replicate runs were studied. Functional groups of pectin were studied using Fourier Transform Infrared spectrophotometry. The degree of esterification and emulsifying properties were determined for assessing the quality of extracted pectin. The result revealed that the extraction conditions strongly affected both

the yield and the quality. The condition of pH 2.5, 110°C, and 30 minutes had the highest degree of esterification of 94.13 % but low yield (1.42%). On the other hand, under the conditions of pH 1.0, 110°C, and 135 min extract time had the highest yield of 3.85% (about 76.6% of pectin content) however the degree of esterification is low at 43.25 %. Two mathematical models were proposed for yield and number of ester groups. The result will be used for the selection of high-quality pectin to produce insoluble hydrogel for pollution treatment in the future.

Keywords: *Eichhornia crassipes*; high-quality pectin; optimum condition; water hyacinth.

INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is a multi-season water plant in the Pontederiaceae family. It can survive



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under many water quality conditions, quickly breed, and distribute to many water bodies worldwide, causing serious problems, particularly blocking water transportation (Carlini *et al.*, 2018). Water hyacinth was currently managed by mechanical harvesting, composting, and handicrafts (Weiping *et al.*, 2018) or left on the river bank, which was not efficient enough due to the large volume and no value-added product to generate business investment. Therefore, finding a way to get rid of water hyacinth and convert it into a high-value product was necessary for incentive management. Its cell walls generally contain many molecularly linked polysaccharides, including cellulose, hemicellulose, and pectin, which can be used in many industries (Audenhove *et al.*, 2021). Optimum extraction conditions however are necessary for these products.

Response Surface Methodology (RSM) is a process dealing with numerous parameters and possible interactions resulting in the desired response (Hundie, 2020). Optimization extraction process when numerous parameters and possible interactions may impact the desired response, especially, to study optimum conditions for extracting pectin from raw materials (Hundie, 2020). Optimizing extraction processes for many raw materials directed by RSM are widely reported, such as the optimization variables of durian rind and coconut husk for pectin extraction (Baothong, 2018), citrus fruit peel (Shukla *et al.*, 2014), and sunflower heads (Ma *et al.*, 2020). However, RSM-based models are only for a restricted range of input parameters and thus, restrict the use for non-linear behavior. It should be noted that in these extraction

processes, the output usually is influenced by many input variables (Peng *et al.*, 2020). Thus, this model is suitable for examining the response to non-linear variables (Pasandide *et al.*, 2017).

The degree of esterification (%DE) was used to consider pectin quality (Robledo and Vázquez, 2023). In the past, pectin was found in water hyacinth since 1989 about 5% of pectin content (Naohara and Ishii, 1989). However, an appropriate extraction method was not reported. Recently, the %DE value of pectin extracted from water hyacinth of $62.26\% \pm 1.32$ was reported under one condition (Jariyapamornkoon *et al.*, 2023). However, it is not high enough for environmental applications. Therefore, it is necessary to find other optimum conditions for extracting pectin from water hyacinth. Generally, hot mineral acid conditions were used to extract pectin because of its high extraction yield and low cost (Audenhove *et al.*, 2021). The most important factors for extracting pectin from agricultural plant waste were pH, temperature, and extraction time. Generally, higher extraction yields are found under harsher conditions such as lower pH, longer extraction time, and higher temperatures (Audenhove *et al.*, 2021) because the links in the side chain of neutral sugar are more acidic than those of the pectin backbone. The high-quality pectin is considered from the number of carboxylic groups that are esterified with alcohol. If the percentage of esterified carboxyl groups is more than 50%, the pectin is referred to as a high degree of esterification pectin (HEP). Whereas the percentage is less than 50%, this pectin is called a low-degree esterification pectin (LEP) (Narasimman and Sethuraman, 2016). Pectin is a

biopolymer that consists of three main domains, namely homogalacturonan and rhamnogalacturonan I, II. The homogalacturonan is a linear polymer consisting only of α -1,4-linked galacturonic acid units which can be methyl-esterified on C-6 and O-acetylated on O-2 or O-3. The percentage of methyl-esterified galacturonic acid unit is expressed as %DE.

There is much research using pectin for environmental protection, such as using pectin hydrogel to absorb metals and heavy metals in wastewater (Wang *et al.*, 2019). The commonly used pectin for adsorption is the LEP type because it has a higher adsorption capacity than the HEP type (Sabando *et al.*, 2023). Because the adsorption of heavy metals by the pectin hydrogel occurs in the carboxyl group (Said *et al.*, 2023). Therefore, if the pectin hydrogel has a high ester group, it will have a lower active site for heavy metal adsorption (Said *et al.*, 2023).

However, it was found that LEP pectin hydrogel has a higher water solubility than HEP because the carboxyl groups on the surface of the pectin polymer matrix could react with water molecules in the dispersion medium and dissolve (Sabando *et al.*, 2023). Therefore, fewer free carboxyl groups on the surface of the pectin hydrogel, and higher water solubility appeared (Said *et al.*, 2023), resulting in the inability to use it in wastewater. Therefore, HEP pectin hydrogel with less solubility will be used as an alternative in heavy metal adsorption. The HEP waste after several times use and removal of the metal is biodegradable and does not cause environmental problems. For that reason, the objective of this research is to study

the new raw material (water hyacinth) and suitable extraction methods to obtain the higher esterification pectin with high ester groups to produce less soluble hydrogel. Multiple linear regression statistics will be applied for the prediction of extraction yield and quality of pectin, which will be handy for future use.

MATERIALS AND METHODS

A. Water hyacinth preparation

Water hyacinth was randomly collected from the drainage canal of Udon Thani municipal, Udon Thani province, Thailand for 5 kg in September 2017. A mature plant with a stem longer than 30 centimeters was collected. Roots were removed and then the stem and leaves were coarsely chopped by hand to the size of about 5 centimeters and blended without adding water in a blender (Phillip model HR2115) for 3 minutes to fine particles. The mixture was preserved at 4°C in a refrigerator for less than three days for pectin extraction.

B. Pectin extraction

The 100 g dried weight equivalent samples of water hyacinth were extracted according to a 3D response surface plot by Box-Behnken design (BBD). Three input parameters were studied including pH, water temperature, and extraction time. Seventeen runs were suggested by the program as shown in *Table 1*. Runs #2, #16 and #7, #9, #11 were replicates of some random conditions. The pH of the solution was adjusted using conc. HCl (AR grade, RCI Labscan), the temperature was controlled by a thermostat (EGO company model 55.13262.130, Thailand), and the extraction time was 30-240 minutes.

Table 1 – RSM suggesting conditions for pectin extraction

pH	Variables		Runs
	Temperature (°C)	Time (min)	
1.0	50	135	1
1.0	80	30	3
1.0	80	240	15
1.0	110	135	12
2.5	50	30	4
2.5	50	240	8
2.5	80	135	7
2.5	80	135	9
2.5	80	135	11
2.5	110	30	13
2.5	110	135	17
2.5	110	240	6
4.0	50	135	2
4.0	50	135	16
4.0	80	30	14
4.0	80	240	10
4.0	110	135	5

After extraction, the samples were filtered through a cheesecloth; 95% ethanol was added at the ratio of 1:1 (v/v) and incubated at 4°C for 12 hours. The filtrates were then centrifuged at 10,000 rpm for 15 minutes using HAEMATOKRIT Premiere model XC-3012. The precipitate was washed with 70% ethanol and the precipitate was collected. The pectin was dried in a vacuum oven (France Etuves model XLF020) at 60°C under vacuum overnight. The pectin precipitate was grounded and sieved using standard sieve no.60 given the diameter particle lesser than 250 µm for further experiments. The yield of pectin was calculated by Equation 1 as follows (Wai *et al.*, 2010):

$$\%yield = \frac{\text{mass of extraction pectin} \times 100}{\text{mass of dried water} \square \text{yacint} \square} \quad (1)$$

C. Characterization of the pectin

The pectin was analyzed by Fourier transform infrared (FTIR) spectrophotometry in the wavenumber

range of 4000-800 cm⁻¹ (Perkin Elmer, Spectrum Two, USA) using a KBr pellet method. Standard pectin AR grade with known DE values of 63.0 – 66.0 % was obtained from LOBA Chemie (Mumbai, India).

D. Quality of pectin

The quality of pectin was assessed using two methods: 1) the emulsifying properties and 2) the degree of esterification.

1) The Emulsifying property was evaluated according to the method described by Dalev and Simeonova (1995). Emulsifying activity (EA) was calculated by Equation 2:

$$EA (\%) = \frac{ELV \times 100}{Wv} \quad (2)$$

where Wv is the whole volume of the solution and ELV is the emulsified layer volume

Emulsion stabilities after 1 and 30 days of storage at 4 and 23°C were calculated using the Equation 3:

$$ES (\%) = \frac{EVEr \times 100}{VEi} \quad (3)$$

where VEr and VEi are the emulsified layer volumes after centrifuging and the initial emulsified layer volumes, respectively.

2) The degree of esterification (%DE) of pectin extracted from water hyacinth. The %DE of pectin was analyzed by the titrimetric method (USP NF21, 2023). The %DE was calculated by Equation 4 as previously described by Santos *et al.* (2013):

$$\text{Degree of esterification (\% DE)} = \frac{V_2 \times 100}{V_1 + V_2} \quad (4)$$

where V₁ is the volume of the initial titer and V₂ is the volume of the final titer

E. Statistical analysis

The data were analyzed statistically using Design-Expert® software, version 13, Stat-Ease, Inc., Minneapolis, MN, USA, www.statease.com. Multiple linear regression (MLR) was used to estimate the relationship between the pectin extraction yield, pectin quality, and the extraction conditions. ANOVA was used to analyze the variables of the extraction process.

RESULTS

The results and discussions were separated into three parts including: A) The relationship between pectin yield and the extraction variables, B) The characterization of the pectin and C) The relationship between pectin quality and the extraction variables.

The details of each step are given below.

A. The relationship between pectin yield and the extraction variables

The pectin extraction yields from water hyacinth are shown in *Table 2*. The control application runs showed similar yield and %DE within the groups (#2, #16) and (#7, #9 and #11). The highest yield was 3.85 % in run #12 (pH 1.0, temperature 110°C, and extraction time 135 min). The extraction yield of pectin was significantly related to the pH. It was found that lower pH resulted in a trend of higher percentage yield ($R^2=0.4558$) (*Figure 1*). With increasing extraction time, the trend of percent yield slightly increased ($R^2=0.098$) at the range of 1.5-2.0% yield and the temperature did not affect the yield. In addition, the hydrogen ions from hydrochloric acid activated the hydrolysis of pectin from proto-pectin

and improved the efficacy to precipitate pectin due to their higher relevance for cations which stabilizes the pectin molecule (Sandarani, 2017).

This result clearly shows that the extraction yield is increased at lower pH as the concentration of hydrogen ions penetrates the polymer structure resulting in better separation of pectin (Yapo, 2009). According to the results of this study, it was found that the pectin extraction efficiency from water hyacinth was as high as 76.6% compared with the 5% content by Naohara and Ishii (1989).

The results from *Table 2* were statistically analyzed by multiple factor linear regression and found that the equations with uncoded factors for extract yield prediction were given in *Equation (5)* as:

$$\begin{aligned} \%yield = & 3.14347 - 2.52702 X_1 + \\ & 0.0143234 X_2 + 0.0215374 X_3 + \\ & 0.522222 X_1^2 + 0.000100000 X_2^2 - \\ & 0.00000340136 X_3^2 - 0.00522222 X_1 X_2 - \\ & 0.00179365 X_1 X_3 - 0.000176984 X_2 X_3 \end{aligned} \quad (5)$$

where X_1 , X_2 , and X_3 are the pH, temperature and extraction time, respectively.

The R-square of yield *Equation (5)* was 0.86. The total extraction yield reflects the extraction efficiency of pectin (Dang *et al.*, 2014; Moonsoor *et al.*, 2001; Singthong *et al.*, 2004). The highest pectin yield was extracted at pH 1.0, 110°C for 135 min (run #12) at 3.85 % w/w. When substituting the values from run 12 to the *Equation (5)*, the result was 3.33% which is close to the experiment yield of 3.85%. This revealed that *Equation (5)* could predict the pectin yield.

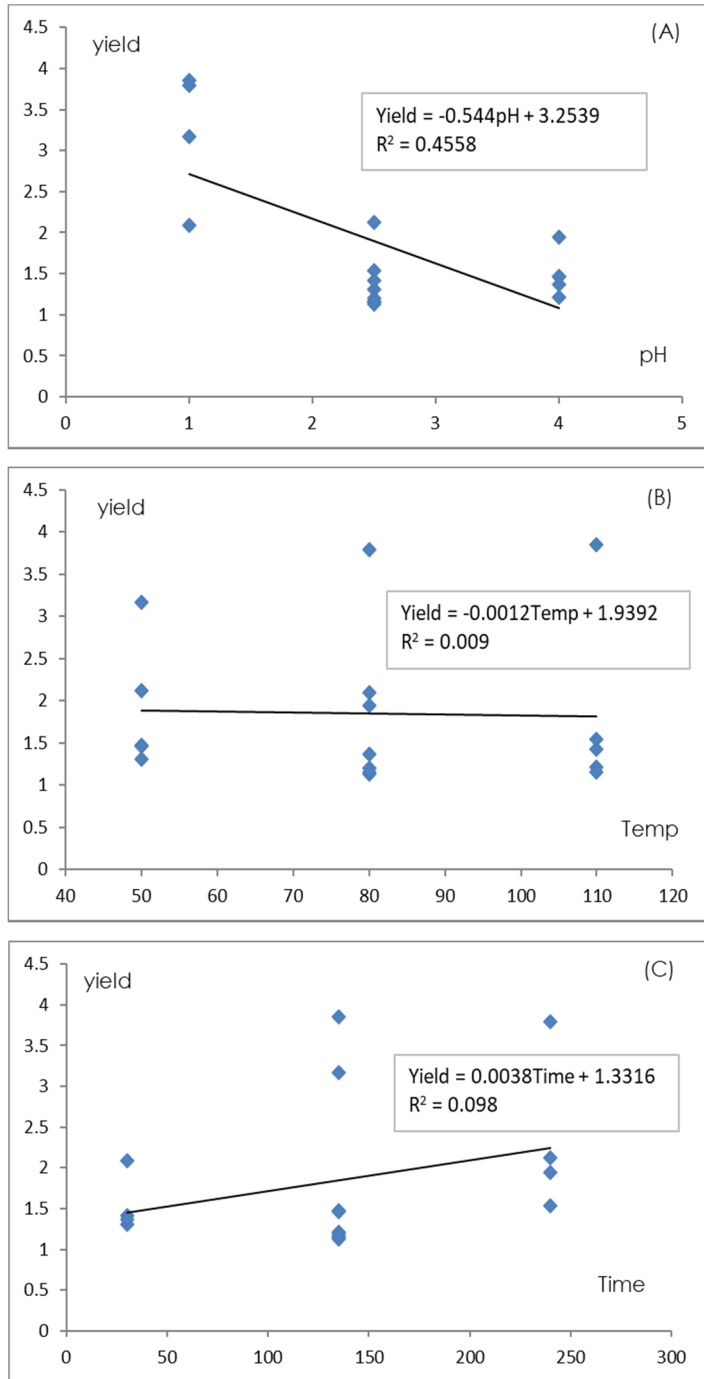


Figure 1 – Relationship between extraction yield of pectin extracted from water hyacinth and (A) pH value, (B) temperature and (C) extraction time

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Table 2 – Yield and DE of each condition of pectin extraction

Runs	Variables			Response	
	pH	Temperature (°C)	Time (min)	Yield (%)	DE (%)
1	1.0	50	135	3.17	0
3	1.0	80	30	2.09	80.51
15	1.0	80	240	3.79	0
12	1.0	110	135	3.85	43.25
4	2.5	50	30	1.31	54.44
8	2.5	50	240	2.12	63.44
7	2.5	80	135	1.13	63.22
9	2.5	80	135	1.15	66.10
11	2.5	80	135	1.20	66.90
13	2.5	110	30	1.42	94.13
17	2.5	110	135	1.16	53.68
6	2.5	110	240	1.54	0
2	4.0	50	135	1.47	38.48
16	4.0	50	135	1.46	38.66
14	4.0	80	30	1.37	67.15
10	4.0	80	240	1.94	60.82
5	4.0	110	135	1.20	53.44

Considering the appropriate model for extraction yield, both quadratic and cubic models were significant ($p < 0.5$) in response to the extraction. The quadratic model was suggested although the cubic model was aliased (Table 3). The F-value and p-value of the extraction model were 13.59 and 0.0012, respectively, implying that the model was significant. The adequate precision ratio was 11.418, moreover, indicates an adequate signal. This model can be used to navigate the design space of extraction yield.

The influence of process variables over the extraction yield was graphically plotted using a 3D response surface plot. It was generated by maintaining two factors that were varied in their ranges (Masmoudi *et al.*, 2008; Thirugnanasamdham *et al.*, 2014) and the result of extraction yield is depicted in Figure 2.

The effect of extraction variables on the pectin extraction yield was graphically plotted using 3D response

surface plots displayed in contour lines and color bands as shown in Figure 2. The plots were generated by maintaining two factors (pH and time in this case), that were varied in their range (Masmoudi *et al.*, 2008; Thirugnanasamdham *et al.*, 2014). From Figure 2, the sequence of yield from low to high is blue, green, yellow, and red, respectively. The extraction yield therefore was greatly influenced by pH and extraction time. The highest yield from Figure 2 (the reddest) was at pH 1.0 and 240 min time in run #15. However, the experimental yield from run #15 was 3.79% compared to run #12 of 3.85% yield. This result may be due to the combination of concurrent phenomena occurring during pectin extraction with a long time and low temperature because of the release of sugar as a product of pectin hydrolysis and degradation by heat (Oliveira *et al.*, 2016). This could be explained by the degrading of the pectin polymer which affects the pectin yield

decrease (Levigne *et al.*, 2002). These results demonstrated that increasing extraction time and suitable temperature increased the extraction yield. The optimum conditions suggested by the

software are shown in *Figure 3*. This optimum condition of pH 1.00533, temperature 88.3172, and extraction time 237.853, resulted in the highest yield of pectin from water hyacinth.

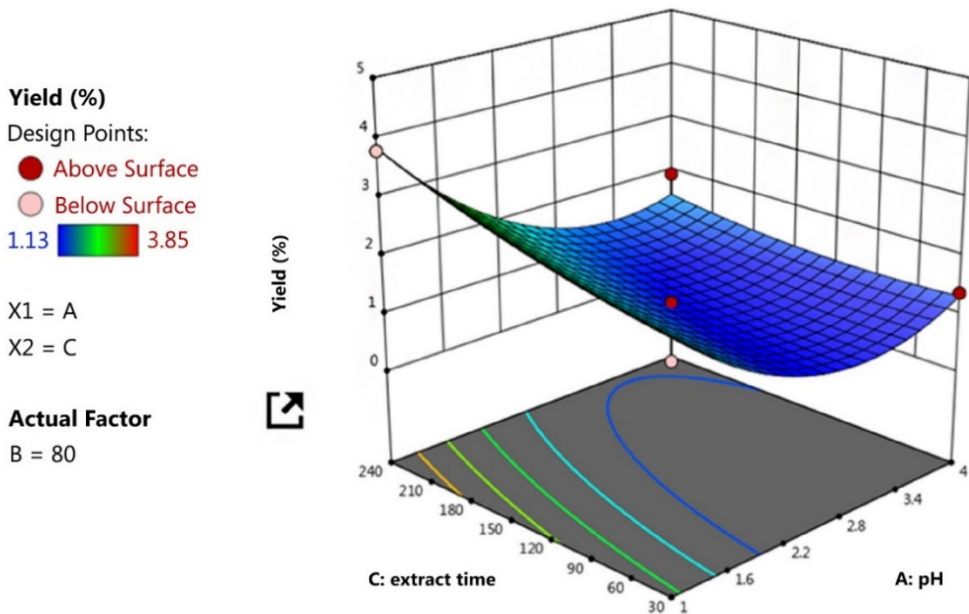


Figure 2 – Three-dimensional (3D) surface plots for a yield of pectin from water hyacinth. The percentage yield of pectin in a sequence from low to high was blue, green, yellow and red, respectively. (The red dot in the middle and every corner of the diagram was the design point appointed by the program to demonstrate that the design of the experimental conditions used in this study obtained from maximum values, median and minimum values of the factors to be studied)

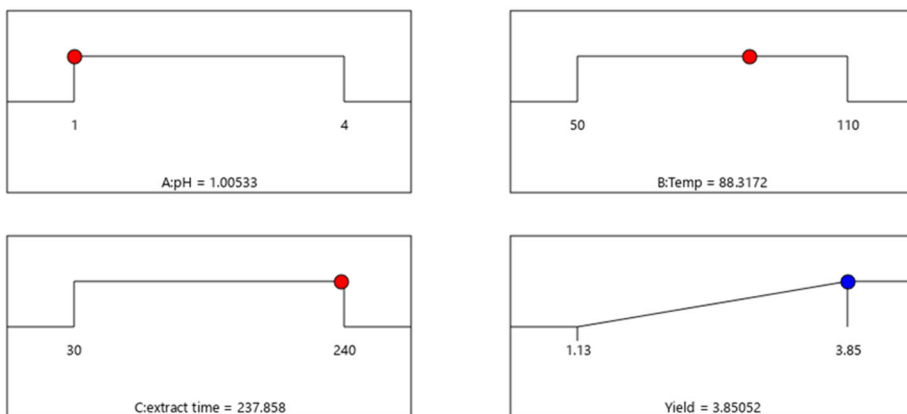


Figure 3 – The highest extracted pectin yield under an optimum condition of pH, temperature and time

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Table 3 – Sequential model sum of squares and ANOVA for response surface linear model of extraction yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean	56.93	1	56.93	-	-	
Linear	7.25	3	2.42	5.16	0.0145	
2FI	0.6592	3	0.2197	0.4044	0.7531	
Quadratic	4.71	3	1.57	15.23	0.0019	suggested
Cubic	0.7151	3	0.2384	137.78	0.0002	aliased
Residual	0.0069	4	0.0017	-	-	
Total	70.27	17	4.13	-	-	
Model	12.62	9	1.40	13.59	0.0012	significant
A-pH	5.97	1	5.97	57.87	0.0001	
B-Temp	0.0003	1	0.0003	0.0030	0.9576	
C- time	1.28	1	1.28	12.41	0.0097	
AB	0.2209	1	0.2209	2.14	0.1867	
AC	0.3192	1	0.3192	3.10	0.1219	
BC	0.1190	1	0.1190	1.15	0.3183	
A ²	4.04	1	4.04	39.17	0.0004	
B ²	0.3289	1	0.3289	3.19	0.1173	
C ²	0.0973	1	0.0973	0.9432	0.3638	
Residual	0.7220	7	0.1031	-	-	
Lack of Fit	0.7151	3	0.2384	137.78	0.0002	significant
Pure Error	0.0069	4	0.0017	-	-	
Cor Total	13.34	16	-	-	-	
Std. Dev.			0.3212			
Mean			1.83			
C.V. %			17.55			
R ²			0.9459			
Adjusted R ²			0.8763			
Predicted R ²			0.8416			
Adeq Precision			11.4182			

B. Characterization of the pectin

Functional groups were analyzed by Fourier Transform Infrared (FTIR) spectrophotometry and the results of the standard and run #13 are shown in *Figure 4 (A)* and *(B)*, respectively. Its spectra of pectin from water hyacinth were generally similar to the standard pectin in *Figure 4 (A)*.

The spectra were determined to affirm the functional groups of extracted pectin. The %DE was estimated by the characteristic peaks of ester groups in the spectra (Begum *et al.*, 2014; Kyomugasho *et al.*, 2015). Wavenumber

range of 950 and 1200 cm⁻¹ as the “fingerprint” region of carbohydrates pectin groups (ether and cyclic C-C bond) (Masmoudi *et al.*, 2008; Santos *et al.*, 2013).

Similar spectra of the standard pectin and the water hyacinth extract in the “fingerprint” region implied that the extracts were pectin. Some carbonyl peaks at 1460 - 1592 cm⁻¹ and 1683 - 1702 cm⁻¹ were related to the free and esterified carboxyl groups, respectively (Thirugnanasambandham *et al.*, 2014). The increase in the intensities and peak area of the esterified carboxyl groups will

also increase the %DE (Oliveira *et al.*, 2016). The peak at 1700 cm^{-1} was ascribed to the C=O stretching of methyl esterified uronic carboxyl group.

This free polysaccharide represents this shift. Determination of the %DE value can be done using the peak area at 1702 cm^{-1} (free carboxyl group) and 1460 cm^{-1} (esterified group) (Begum *et al.*, 2014).

The peak at 2841 cm^{-1} showed the methoxyl group and was ascribed to the quality of pectin.

C. Relationship of pectin quality and the extraction variables

The quality of extracted pectin from water hyacinth is shown in *Table 2*.

The DE was plotted against pH, temperature, and time in *Figure 5*. It was found that increasing the extraction time resulted in a trend of decreasing DE ($R^2=0.3077$).

On the contrary, increasing pH and temperature had the trend of slightly increasing DE ($R^2=0.0676$ and 0.0204 , respectively). The %DE of pectin was investigated according to BBD (17 runs) and its results are shown in *Table 2*. The highest DE was 94.13 % in run #13 from the extraction condition of pH 2.5, temperature 110°C, and extraction time of 30 min.

In addition, some conditions, such as, run #1, #6, and #15 with a long extraction time showed different characteristic peaks other than the standard pectin assuming that they were not pectin (DE=0).

The degree of esterification was analyzed by multiple factor linear regression and found that the equations with uncoded factors for DE prediction were given as *Equation (6)* below:

$$DE = -164.494 + 38.7010 X_1 + 4.64162 X_2 - 0.0322874 X_3 - 9.82056 X_1^2 - 0.0235708 X_2^2 + 0.000696259 X_3^2 + 0.0775556 X_1 X_2 + 0.117746 X_1 X_3 - 0.00818492 X_2 X_3 \quad (6)$$

where X_1 , X_2 , and X_3 are the pH of the solution, temperature, and extraction time, respectively.

The R-square of *Equation (6)* was 0.97. The DE reflects the extraction quality of pectin. When substituting the values from run #13 in the quality *Equation (6)*, the calculated DE was 99.06% and is comparable to 94.13% from the actual value. If high methoxyl pectin (HMP) has a degree of esterification values higher than 50%, then many conditions in this study could produce HMP for example run #13, #3, #8 etc.

The 2FI and quadratic models were the appropriate model for the quality of pectin from water hyacinth. The cubic model was aliased, which implies that it cannot be selected (*Table 4*).

The 2FI and quadratic model were significant with p-values of 0.0266 and 0.0256, respectively. The adequate precision ratio was 12.3274 indicating an adequate signal and was possible to navigate the design space of pectin quality.

The effect of extraction variables on the %DE is depicted in *Figure 6*. It demonstrates the effect of extraction time and temperature on the %DE.

The highest DE value of 94.13% was found when the pH of 3.2 and the extraction time was at 135 min and 80°C. Indeed, similar studies have found behaviors for the DE value as a function of extraction conditions (Yapo *et al.*, 2007).

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Other research (Baothong, 2018; Oliveira *et al.*, 2016) showed the extraction of pectin from each material using high pH values produced pectin with higher DE values. This can be described as the higher de-esterification of the polygalacturonic chain at very low pH conditions.

The results revealed that extracted pectin from water hyacinth with high %DE values where extraction time not longer than 30 minutes in runs #3 and #13, which the %DE values higher than 80%. In run #14, even though the pH

value was high (4.0) and the extraction time was low, the %DE value was still considered high (67.15%). Although only limit conditions were tested, the optimum extraction conditions at a short time was likely to respond to a high %DE value.

Figure 7 shows the optimum condition of each parameter calculated by the RSM software to achieve a high %DE. The optimum condition was 2.59, 99.80, and 42.67 for pH, temperature, and extraction time, respectively. The %DE at the optimum condition was 96.87%.

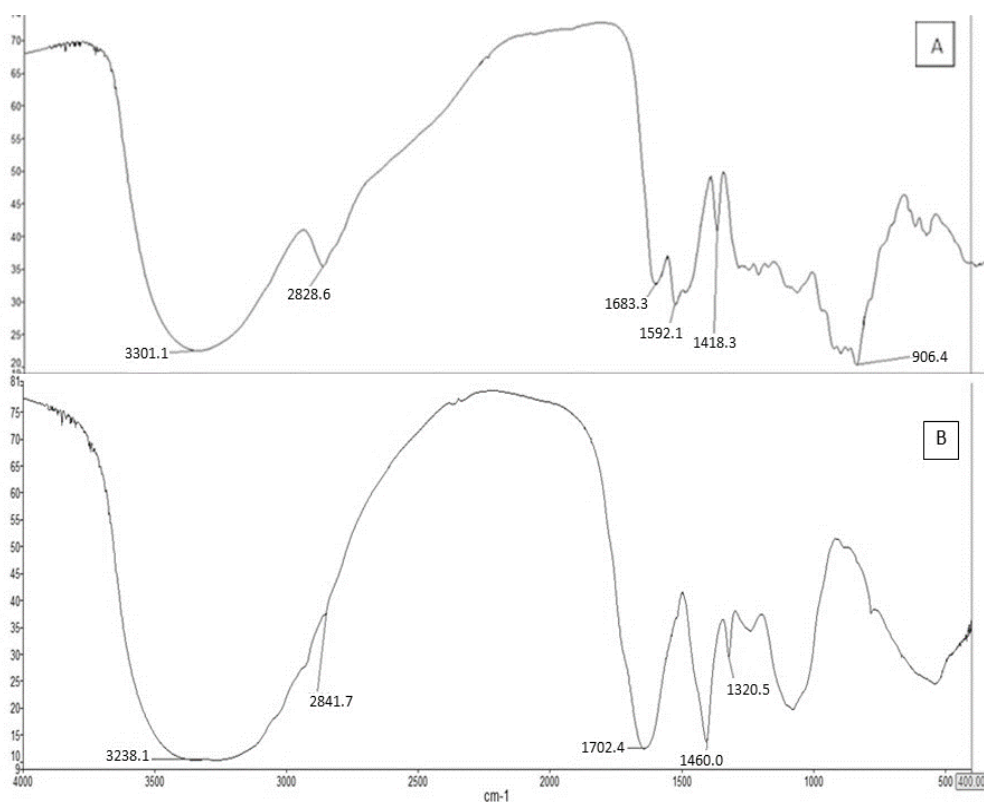


Figure 4 – FTIR spectra of water hyacinth pectin obtained at (A) standard pectin, (B) run #13 (The highest quality pectin)

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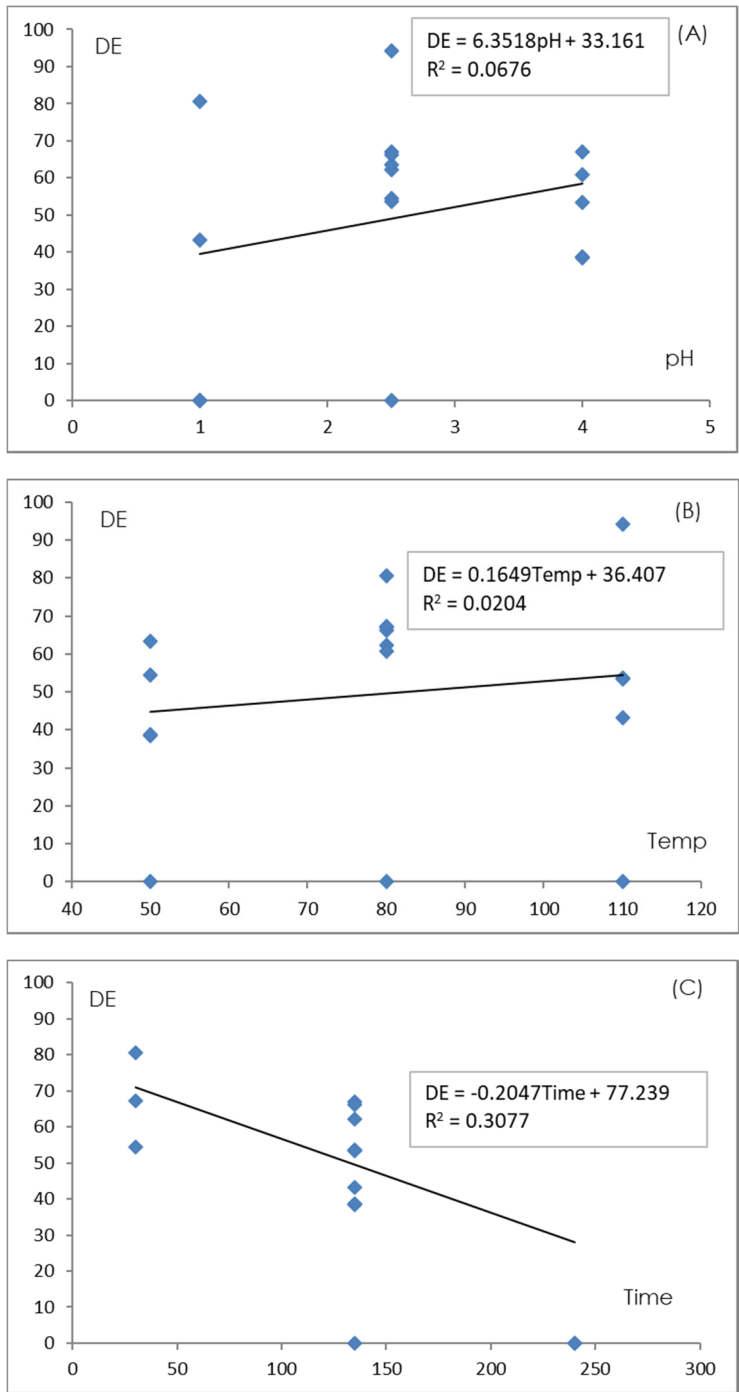


Figure 5 – Relationship between DE of pectin extracted from water hyacinth and (A) pH, (B) temperature and (C) extraction time

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Table 4 – Sequential model sum of squares and ANOVA for response surface linear model of %DE

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean	45474.29	1	45474.29	-	-	
Linear	5000.27	3	1666.76	3.00	0.0694	
2FI	4234.70	3	1411.57	4.72	0.0266	suggested
Quadratic	2137.28	3	712.43	5.83	0.0256	suggested
Cubic	840.23	3	280.08	73.06	0.0006	aliased
Residual	15.33	4	3.83	-	-	
Total	57702.10	17	3394.24	-	-	
Model	11372.25	9	1263.58	10.34	0.0028	significant
A-pH	1155.12	1	1155.12	9.45	0.0180	
B-Temp	148.44	1	148.44	1.21	0.3069	
C- time	3696.71	1	3696.71	30.25	0.0009	
AB	200.08	1	200.08	1.64	0.2415	
AC	1375.67	1	1375.67	11.26	0.0122	
BC	2658.95	1	2658.95	21.75	0.0023	
A ²	1064.86	1	1064.86	8.71	0.0214	
B ²	949.96	1	949.96	7.77	0.0270	
C ²	46.05	1	46.05	0.3767	0.5588	
Residual	855.56	7	122.22			
Lack of Fit	840.23	3	280.08	73.06	0.0006	significant
Pure Error	15.33	4	3.83			
Cor Total	12227.81	16				
Std. Dev.			11.06			
Mean			51.72			
C.V. %			21.38			
R ²			0.9300			
Adjusted R ²			0.8401			
Predicted R ²			0.8416			
Adeq Precision			12.3274			

The emulsifying properties of the highest %DE pectin from Run #13, which was extracted under optimal extraction conditions were studied. The emulsifying activity (EA) and emulsion stability (ES) of the highest %DE pectin are shown in Table 5. This research shows that the emulsion stability had a high level under the two different tested storage temperatures, emulsion stability levels at 4°C after 1 day and 30 days, were 92.3% and 87.4%, respectively. But emulsion stability levels at 23°C after 1 day and 30 days, decreased to 79.6% and 51.2%, respectively. Therefore, the factors that

greatly affect the emulsifying activity of pectin extracted from water hyacinth were temperature and time (Bichara *et al.*, 2016; Hosseini *et al.*, 2016).

DISCUSSION

The response of pectin extraction in this case is a non-linear combination condition of three parameters (pH, temperature, and extraction time). Using the response-surface model was suitable and could reduce the number of wet experiments (Pasandide *et al.*, 2017; Peng *et al.*, 2020).

Percentage pectin yields extracted from raw materials are significantly varied in the range of 1.42-71.5% as shown in *Table 6*. Although pectin could be extracted from water hyacinth, the extraction yield was comparatively lower than other materials. This reflects the fact that the cell walls of water hyacinths contain very low (about 5%) pectin (Naohara and Ishii, 1989). The quality of the pectin extracted from water hyacinth on the contrary had the highest %DE of 94.13%. This number was higher than other research, which extracted pectin from water hyacinth with a %DE value of 62.26% in conditions with a pH of 2.0, temperature of 80°C, and extraction time of 60 minutes. However, the extraction time was too long. The ester group in the pectin chain is converted to a carboxylic group. The -CH₃ of the ester group will

be replaced by a hydrogen atom and the %DE value will be lower (Ji-u and Neamsorn, 2022).

High-quality pectin can be used in many industries. The price of high-quality pectin was \$99.17/Kg for Laboratory and pharmaceutical grades (Happi *et al.*, 2008). Furthermore, a high degree of esterification provided the active sites for cross-linked to other cations such as calcium and boron to form stable (hard to be dissolved) hydrogel which can be used as a base for heavy metal adsorption. Thus, the optimum condition for high %DE pectin in this study can improve an adsorbent that is less soluble and can be used for adsorption for wastewater treatment repeatedly and efficiently. Therefore, it is another alternative way to manage water hyacinth problems in water sources.

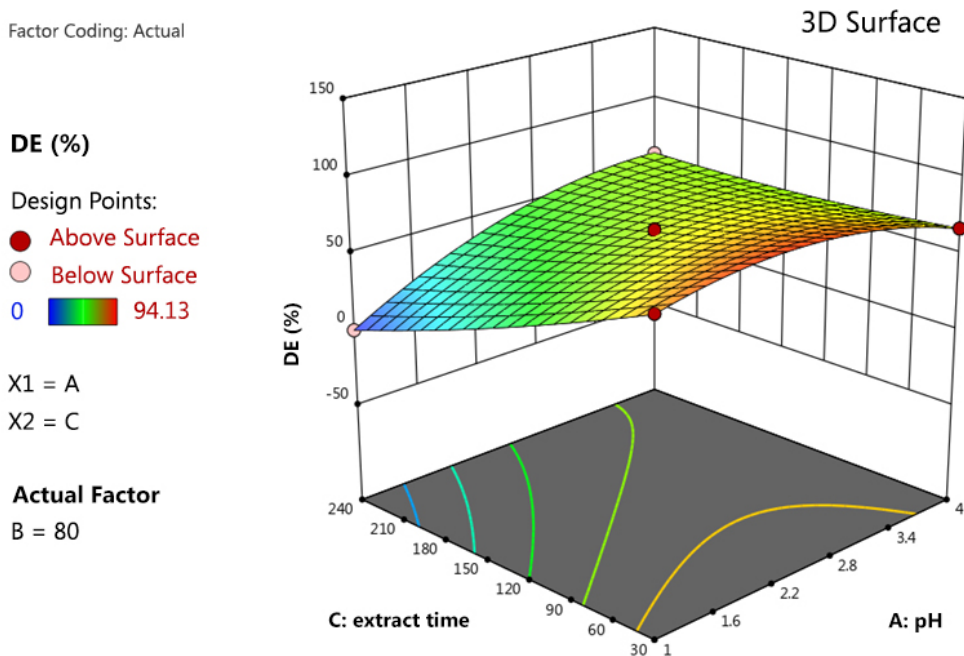


Figure 6 – Three-dimensional (3D) surface plots for the %DE from water hyacinth. The %DE in a sequence from low to high was blue, green, yellow and red, respectively.

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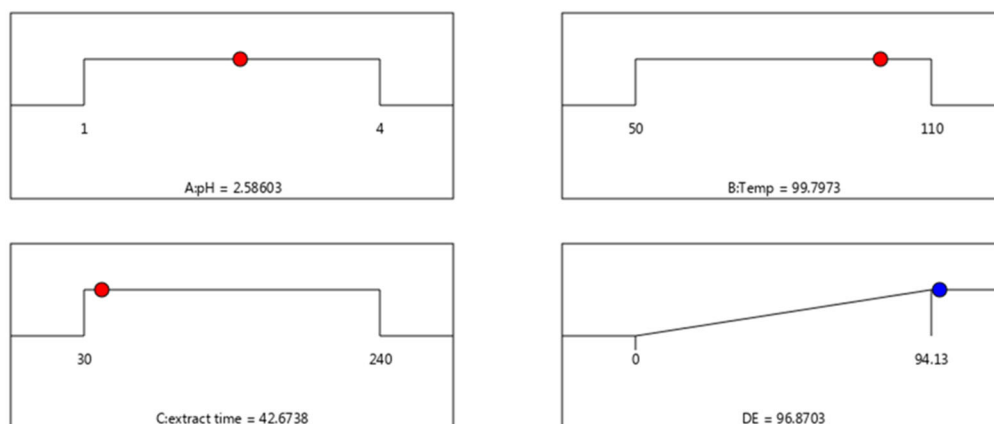


Figure 7 – The highest %DE under an optimum condition of pH, temperature and time

Table 5 – Emulsifying activity and emulsion stability (%v/v) of pectin solutions (0.5%w/w)

Storage time (day)	Emulsion activity (%)	Emulsion stability (%)			
		1	23	4	30
Temperature (°C)		4	23	4	23
Pectin Run #13*	42	92.3	79.6	87.4	51.2

* Pectin with the highest %DE (at the temperature 110°C, extraction time 30 min and pH 2.5)

Table 6 – Yield and DE of pectin extracted from each material

Material	%DE	%Yield	References
Water hyacinth	94.13	1.42	This study
Water hyacinth	62.26	5.42	Jariyapamornkoon <i>et al.</i> (2023)
Broccoli	76.50	71.50	Christiaens <i>et al.</i> (2011)
Durian rind mixed with coconut coir	65.80	9.10	Baothong (2018)
Dragon fruit	-	7.50	Thirugnanasambandham <i>et al.</i> (2014)
Pomegranate peel	47.43	25.96	Pereira <i>et al.</i> (2016)
Lemon peel	-	11.21	Ciriminna <i>et al.</i> (2016)

CONCLUSIONS

The pectin extracted from water hyacinth was classified as high quality because of the high %DE. Extraction conditions affected both the extraction yield and quality of pectin. For future use, multiple linear regression models for the extraction yield and quality of pectin were proposed. The extraction yield was affected by pH and extraction time. The degree of esterification is affected by extraction time and pH. The optimum

condition given high-quality pectin from water hyacinth of 94.13% DE was pH 2.5, 110°C, and an extraction time of 30 min. The high-quality pectin can be applied in many industries including repeatable desorption hydrogel granules for wastewater treatment.

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