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Natural Deep Eutectic Solvent-Assisted Pectin Extraction from Pomelo Peel Using Sonoreactor: Experimental Optimization Approach

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Abstract: Background: Natural deep eutectic solvents (NADESs) can be used for extracting a wide range of biomaterials, such as pectin. This study introduces a new generation of natural solvents for pectin extraction which could replace the conventional solvents in the food industry. Methods: In this study, NADESs were used for pectin extraction from pomelo (*Citrus grandis* (L.) Osbeck) peels using a sonoreactor. Definitive screening design (DSD) was used to screen the influence of time, temperature, solid/liquid ratio, and NADES/water ratio on the pectin yield and degree of esterification (DE). Results: The primary screening revealed that the best choices for the extraction were choline chloride–malonic acid (ChCl-Mal) and choline chloride–glucose–water (ChCl:Glc:W). Both co-solvents yielded 94% pectin and 52% DE after optimization at 80 °C, with 60 min of sonication, pH < 3.0, and a NADES-to-water ratio of 1:4.5 (*v*/*v*). Morphological screening showed a smooth and compact surface of the pectin from ChCl-Mal where glucose-based pectin had a rough surface and lower DE. Conclusions: NADESs proved to be promising co-solvents for pectin extraction with a high degree of esterification (>55%).

Keywords: pectin; natural deep eutectic solvent; *Citrus grandis*; definitive screening design; pomelo; extraction

1. Introduction

Plant cell walls are rich in pectin, which is the most complex natural structural family of polysaccharides [1]. It is a polymer constructed by α-(1,4)-bonds between D-galacturonic acid molecules with specific esterification levels [2]. Pectins are classified into two main groups: high methoxyl (HM) and low methoxyl (LM) pectins. HM pectins have a degree of esterification (DE) of 55–80%, and they gel at low pH in the presence of sugar [3]. On the other hand, LM pectin has a DE value <50% [2].

Malaysia is blessed with tropical weather which generates an extensive and noteworthy variety of edible fruits [4]. Many types of fruits are consumed widely and, consequently, wastes are produced, which would create disposal problems. Pomelo (*Citrus grandis* (L.) Osbeck) is the largest citrus fruit, and is a potentially good source of pectin from the non-edible part of the fruit [2].

Pectin extraction is usually performed using mineral acids, yielding a reasonable quantity of pectin while also saving time in the process. Nonetheless, pectin extracted by means of this approach is vulnerable. Furthermore, high acidity speeds up corrosion and rust formation of the apparatus, leading to water pollution which contributes to environmental concerns [2].

Pectin is used as a gelling agent and as a polymer that stabilizes many cosmetic and food products [1]. Even though pectin has various applications in the food manufacturing industry, consumers have a negative perception upon learning that strong acids are used in pectin extraction. Hence, from a food safety point of view, many studies were carried out to address this problem in which organic acids were substituted for mineral acids in the pectin extraction process [5]. It was also inferred that deep eutectic solvents (DESs) are an excellent choice for extraction because they are non-volatile at room temperature and non-toxic [6], and the majority of DESs are water-miscible [7] depending on their components. Hydrophobic DESs were also used for industrial processes, for example, as extracting agents for furfural and its derivatives [8] and for separation [9].

Natural deep eutectic solvents (NADESs) are composed of natural ingredients inclusive of amino acids, sugars, or carboxylic acid [10]. The potential suitability of natural deep eutectic solvents (NADESs) was evaluated for pectin extraction in the present study. NADESs are less toxic than DESs, and it was reported that the use of natural biomaterials appears to be an important factor for lowering the cytotoxicity of NADESs. Malic acid, citric acid, and oxalic acid are components of the Krebs cycle and naturally occur in the mitochondria [11]. Citric acid was used as a typical solvent for pectin extraction [12]. Recently, a Box–Behnken design was applied to optimize the extraction of pectin from *Averrhoa bilimbi* (ABP) using DES with a maximum experimental yield of 14.44 wt.% [13]. Liew et al. [2] used citric acid combined with DESs to extract pectin from *Citrus grandis*. At 88 °C and 141 min, the yield of pectin obtained was 39.72 wt.% with a DE% value of 57.56%. The study showed promising results; therefore, we aimed to introduce NADESs as a greener option for pectin extraction.

The targets of the current study were to assess the optimal pectin extraction conditions from pomelo peel using carboxylic acids or sugar-based NADESs. This study aimed to also have a clear perception of the influence of temperature, time of sonication, ratio of the solid, and the ratio of NADES/water on the yield and the degree of esterification (DE%). The frame of the study involved applying various types of NADESs and their raw ingredients in extracting pectin and assessing the morphology of all the acquired extracts to have a clear understanding of the extractive abilities of NADESs. Moreover, compared to current studies, the optimization of pectin extraction aimed to reduce the temperature, time required, and the ratio of NADES used for efficient extraction.

2. Materials and Methods

2.1. Materials

The fruit used in this study, pomelo (*Citrus grandis* (L.) Osbeck), was collected from Pasar Borong Selayang, Selangor, Malaysia. The fruit was of the same ripeness and batch. The fruits were washed with water, peeled, and the peels were cut. Peel samples were dried at 60 °C until a constant weight was achieved. The dried peel was ground into powder (<1000 μm). The powder was conserved in a dry environment in an airtight container. All solvents and chemicals used in this study were of analytical grade.

2.2. NADESs Preparation

All of the NADESs were prepared based on the molar ratio of choline chloride and the hydrogen bond donor (HBD) and all ratios were presented in Section 3.1. The appropriate ratio for the preparation

was taken from the literature [14,15]. Choline chloride (ChCl) and an HBD (of choice) were mixed at a specific molar ratio, and heated and stirred at a speed of 350 rpm and a temperature at 80 °C (Thermo Scientific ™, Waltham, MA, USA) for 2 h until a homogeneous and stable mixture with no visible precipitate was obtained. The water content of all NADESs ranged from 0.43–1.23% (Karl-Fischer titration). Addition of deionized water was included in the synthesis of aqueous NADESs based on a molar ratio with hydrogen bond acceptor (HBA) or salt [16].

2.3. Pectin Extraction

Solvent screening for the extractive capability of pectin was conducted under constant conditions to determine the solvent with the best pectin extraction performance. Next, the selected NADESs were used for definitive screening design optimization.

2.3.1. Screening of the Potential NADESs and Their Individual Components

Firstly, 1.5 g of dried pomelo powder was mixed with each of the prepared NADESs. NADESs were diluted with distilled water at the ratio of 3:1 (NADES–water) while the raw components (glucose, fructose, sucrose, oxalic, citric, malic and malonic acids, and ChCl) were dissolved at the ratio of 1 g of salt to 40 mL of water at the room temperature (25 ± 2 °C). A sonication bath (Branson 3800, Danbury, CT, USA) was used as a sonoreactor for the extraction process and to incubate the mixture at a fixed time (120 min) and temperature (60 °C).

2.3.2. Optimization Using Definitive Screening Design (DSD)

The same quantity of dried pomelo powder was mixed with each HBD, salt solution, and NADES at different solid/liquid ratios. Definitive screening design (DSD) was selected as it allows the estimation of all main effects and shows clear second-order effects. Four factors were selected for the optimization including temperature, sonication time, solid/liquid ratio, and DES/water ratio. The ranges are presented in Table 1. The mixture was placed in the sonoreactor in the designated conditions. The design was obtained and analyzed using Design-Expert®version 11 (Stat-Ease, Inc., Minneapolis, MN, USA)

Table 1. Definitive screening design input for factors affecting pectin extraction. NADES—natural deep eutectic solvent.

No	Factor	Level	
		Low	High
1	X_1: Liquid/solid ratio	10	40
2	X_2: Temperature, °C	60	80
3	X_3: Time, min	60	120
4	X_4: NADES/water ratio	1:6	1:3

2.4. Pectin Yield Determination

After the completion of sonication cycles, the tubes holding the mixture were centrifuged (Thermo Scientific ™, Leicestershire, UK) for 5 min at 1900× g (8 × 50 mL fixed angle rotor). The obtained filtrate was coagulated with 1.5 times the supernatant volume of ethanol (96%) and was allowed to set overnight. The formed pectin was filtered through fabric filters and washed with distilled water followed by ethanol (70%). The obtained participate was dried in a 70 °C drying oven until a constant weight was reached (3–5 h). The pectin yield was computed using Equation (1) [17].

$$Yield\ of\ Pectin\ (wt\ \%) = \frac{m_0}{m} \times 100, \quad (1)$$

where dried pectin weight is expressed as m_0 (g), and the initial powder's weight is m (g).

2.5. Degree of Esterification (DE%) Determination

The quantity of DE is a measure of the number of esterified carboxyl groups as a percentage of the number of galacturonic acid groups. The pectin DE% was assessed using the titration method reported by Liew et al. [2]. Firstly, 0.2 g of the obtained pectin was dampened with ethanol and incubated in a 45 °C water bath, with intermittent shaking, until complete dissolution. To start the titration, phenolphthalein was dropped onto the mixture and titrated against 0.1 N NaOH. The volume was recorded at the endpoint. Then, the solution was brought to neutral with 10 mL of 0.1 N NaOH. The sample was shaken and permitted to stand for 2 h at room temperature for pectin to be de-esterified. NaOH was neutralized with 10 mL of 0.1 N HCl until its pink color vanished. Phenolphthalein was added and the second reading was recorded with 0.1 N NaOH at the first appearance of pink color. The percentage DE was calculated based on the following formula:

$$DE\ (\%) = \frac{Final\ titration\ volume\ (mL)}{Initial\ titration\ volume\ (mL) + Final\ titration\ volume\ (mL)} \times 100. \quad (2)$$

2.6. Analysis of Pectin Samples Using Field-Emission Scanning Electron Microscopy (FESEM)

Field-emission scanning electron microscopy (FESEM, Model: FEI Quanta FEG 650, Thermo Fisher Scientific, Waltham, MA, USA) was used to investigate the surface morphological features such as the porous pattern and surface structure of pectin extracted by NADES. The elemental analysis of sugar–NADES pectin and acid–NADES pectin was carried out using energy-dispersive X-ray spectroscopy (EDS).

3. Results and Discussion

3.1. Screening of the Potential NADESs and the Former Components

The pectin extraction was evaluated in several deep eutectic solvents, their components, and water. The results are shown in Table 2, expressed in terms of yields per gram of powder used for extraction. It can be seen that water resulted in a meager pectin yield compared to all the NADESs used. However, salt only, such as choline chloride (ChCl), had the lowest extractive ability. In contrast, D-malic acid and oxalic acid were the best (60–76%) in comparison with the remaining raw materials in this study. A moderate yield (30–37%) was obtained in the case of fructose < citric acid < malonic acid. When choline chloride was combined with organic acids such as citric acid, malic acid, and oxalic acid, comparable results were achieved. The pectin yield fluctuated between 33% and 45%. On the other hand, ChCl–malonic acid (1:1) yielded 96.4% of the initial weight of the powder. A comparable yield was recorded with ChCl–glucose–H_2O (5:2:5) followed by ChCl–glycerol (1:2). In summary, the highest yield was recorded with ChCl combined with glucose and malonic acid (96.7% and 96.4%).

Table 2. Percentage of pectin yield per gram of powder used in several NADESs and raw components compared with water*.

Entry	Co-solvent	Ratio	Pectin Weight (g)	Pectin Yield (wt.%)
1	ChCl–malonic acid	1:1	1.45 ± 0.05	**96.37** ± 2.00
2	ChCl–fructose–H_2O	5:2:5	1.07 ± 0.05	71.49 ± 1.00
3	ChCl–glycerol–H_2O	1:2:1	0.63 ± 0.02	41.71 ± 1.00
4	ChCl–fructose	5:2	0.44 ± 0.02	29.25 ± 1.00
5	ChCl–sucrose–H_2O	4:1:4	0.78 ± 0.03	51.73 ± 1.00
6	ChCl–glucose–H_2O	5:2:5	1.45 ± 0.05	96.73 ± 2.00
7	ChCl–glucose	5:2	0.76 ± 0.02	50.54 ± 1.00
8	H_2O	-	0.21 ± 0.01	13.73 ± 1.00
9	ChCl–citric acid	1:1	0.65 ± 0.01	43.18 ± 1.00
10	ChCl–malic acid	1:1	0.67 ± 0.02	44.96 ± 1.00
11	ChCl–oxalic acid	1:1	0.49 ± 0.02	32.91 ± 1.00
12	D-Malic acid only	1:1	1.15 ± 0.05	76.53 ± 1.00
13	Oxalic acid	1:1	0.91 ± 0.05	60.56 ± 1.00
14	Fructose	1:1	0.45 ± 0.02	29.80 ± 1.00
15	Malonic acid	1:1	0.56 ± 0.02	37.01 ± 1.00
16	Choline chloride	1:1	0.047 ± 0.00	3.16 ± 1.00
17	Citric acid	1:1	0.52 ± 0.02	34.45 ±1.00
18	Fructose–citric acid	1:1	0.37 ± 0.02	36.97 ±1.00

* The extraction was performed under fixed conditions: 1.5 g of powder, 10 mL–30 mL NADES/water ratio, and 120 min sonication at 60 °C in a sonication bath. The batch was duplicated, and the values are means ± standard deviation (SD).

It was reported that one of the most important parameters that may influence the pectin quantity and characteristics is pH [4]. Therefore, the obtained results may be correlated with the pH of the solution. The pH observed in the cases with highest yield was between 2.0 and 4.0 due to the acidic properties of the co-solvents. At low pH value, the concentration of $[H^+]$ of the solution increases, which leads to lower ionization of the carboxylate groups [18]. The loss of carboxylate groups reduces the repulsion of polysaccharide molecules, which facilitates the gelation properties of pectin, yielding more precipitated pectin at low values of pH [19].

The results are presented in Table 2 as pectin yield (wt.%), but can be a combination of other polysaccharides and sugars which were extracted along with the pectin. The results nonetheless showed that the investigated NADESs had high extraction yields compared to their individual compounds such as salt or HBD. Thus, the NADESs are suitable for applications where polysaccharides have to be dissolved or extracted. Notable is the affinity of the pectin for the acid-based NADES compared to other types of NADESs. The acid-based NADES are not as acidic as HCl; however, they show a much higher yield, indicating their suitability when searching for a NADES to work with polysaccharides [20]. The NADES composition results in a broad range of physical characteristics, which generate different behaviors in the extraction; therefore, they possess a wide range of extractive abilities [21]. The water in NADESs acts as an HBD to form the eutectic mixture. Hence, it is the part of the NADES that is contributing in the hydrogen-bonding network. This was discussed in detail in previous publications by our group [22] and other research groups [23].

Moreover, the data we obtained imply that the organic acid NADESs were the best co-solvents in these tested extractions. These NADESs were reported to be more acidic (pH < 3) by nature compared to sugars or polyalcohol NADESs (pH > 6) [24]. The screening results are consistent with the reported data. Based on the screening, the highest extraction ability was observed with two co-solvents: ChCl–Glc–W and ChCl–malonic acid; therefore, they were selected for optimization by DSD design. The factors were selected based on the current screening results, as well as previously conducted studies by Liew et al. [2] and Raji et al. [17].

3.2. Optimization Using Definitive Screening Design (DSD)

Table 3 shows the results of the experimental design of DSD of both ChCl–Mal and ChCl–Glc. The design was selected as it could provide both screening and optimization at three levels of each parameter without the need of a second optimization.

Table 3. Definitive screening design for the screening of four factors at three levels of pectin extraction using ChCl–malonic acid (M) and ChCl–glucose–water (G).

					(M)		(G)	
Factors	X_1	X_2	X_3	X_4	**Response 1**	**Response 2**	**Response 1**	**Response 2**
Run	**L/S * ratio**	**T * °C**	**t * min**	**NADES/ Water Ratio**	**Yield %**	**DE%**	**Yield Wt.%**	**DE%**
1	40	80	120	1:6	11.25 ± 1.5	49.02 ± 1.75	12.68 ± 1.5	49.52 ± 1.75
2	10	80	60	1:6	14.97 ± 1.5	58.99 ± 1.75	16.32 ± 1.5	59.76 ± 1.75
3	40	60	90	1:6	59.68 ± 1.5	51.09 ± 1.75	72.25 ± 1.5	52.03 ± 1.75
4	40	60	120	1:3	21.49 ± 1.5	56.29 ± 1.75	57.59 ± 1.5	55.55 ± 1.75
5	10	70	120	1:6	5.79 ± 1.5	39.88 ± 1.75	6.40 ± 1.5	40.17 ± 1.75
6	40	80	60	1:4.5	93.93 ± 1.5	62.19 ± 1.75	87.59 ± 1.5	60.89 ± 1.75
7	10	60	120	1:4.5	7.25 ± 1.5	38.22 ± 1.75	4.14 ± 1.5	41.07 ± 1.75
8	10	60	60	1:3	16.64 ± 1.5	57.91 ± 1.75	15.12 ± 1.5	55.76 ± 1.75
9	10	80	90	1:3	32.19 ± 1.5	60.01 ± 1.75	33.73 ± 1.5	59.51 ± 1.75
10	40	70	60	1:3	86.97 ± 1.5	54.76 ± 1.75	84.27 ± 1.5	55.33 ± 1.75
11	25	80	120	1:3	5.09 ± 1.5	53.85 ± 1.75	5.33 ± 1.5	52.01 ± 1.75
12	25	60	60	1:6	38.17 ± 1.5	50.48 ± 1.75	35.68 ± 1.5	49.78 ± 1.75
13	25	70	90	1:4.5	43.31 ± 1.5	47.74 ± 1.75	48.12 ± 1.5	48.12 ± 1.75

* T = temperature, t = time, L/S = liquid/solid ratio.

As seen from Table 3, the maximum pectin yields and DE%, (93.93 wt.%, 62.2%, and 86.97%, 54.8%) were obtained at runs 6 and 10, respectively. Similar results were obtained in the case of ChCl–Glc–W with a lower yield of pectin and a comparable DE% content.

The relationship between the studied factors and the pectin yield was expressed with a second-order equation obtained from Design-Expert® by experimental data analysis. The final coded Equation (3) with ChCl–malonic acid is given as

$$\begin{aligned}\text{Pectin yield (wt\%)} &= 35.8235 + 56.0917\,X_1 + 28.4629\,X_2 + -6.35237\,X_3 \\ &+ 34.0298\,X_4 + -7.6616\,X_1X_2 + -24.4983\,X_1X_3 \\ &+ -153.752\,X_1X_4 + -7.98837\,X_2X_3 + -115.602\,X_2X_4 \\ &+ -53.1371\,X_3X_4 + -13.6278\,{X_1}^2 + -17.802\,X_1X_2X_4.\end{aligned} \quad (3)$$

The degree of esterification DE% is given by

$$\begin{aligned}\text{DE (\%)} = 39.2239 &+ 15.4426\,X_1 + 23.607\,X_2 + -23.4922\,X_3 + 38.7182\,X_4 \\ &+ -4.45193\,X_1X_2 + -5.82252\,X_1X_3 + -60.0954\,X_1X_4 \\ &+ 4.01979\,X_2X_3 + -88.4539\,X_2X_4 + 81.2432\,X_3X_4 \\ &+ 4.95433\,{X_1}^2.\end{aligned} \quad (4)$$

However, in the case of ChCl–Glc–W, the two equations are expressed as

$$\begin{aligned}\text{Pectin yield (wt\%)} &= 32.2677 + 75.4426\,X_1 + 62.1528\,X_2 + -18.0211\,X_3 \\ &+ 60.9422\,X_4 + -23.7504\,X_1X_2 + -32.3432\,X_1X_3 \\ &+ -220.358\,X_1X_4 + -4.91571\,X_2X_3 + -257.091\,X_2X_4 \\ &+ 22.2387\,X_3X_4 + -13.3185\,{X_1}^2,\end{aligned} \quad (5)$$

$$\begin{aligned} \text{DE}\ (\%) = \ 41.2793 \quad & + 9.11799\ X_1 + \ 9.51682\ X_2 \ + \ -14.8729\ X_3 \ + 31.1046\ X_4 \\ & + \ -2.0271\ X_1X_2 + \ -0.939875\ X_1X_3 \ + \ -32.382\ X_1X_4 \\ & + 0.621687\ X_2X_3 + \ -29.7551 \ X_2X_4 + \ 45.1128\ X_3X_4 \\ & + 4.56781\ {X_1}^2. \end{aligned} \quad (6)$$

Analysis of variance (ANOVA) of the first response, pectin yield (Table 4), revealed that the model F-value (936.46) implies a significant model. In the current design, X_1, X_2, X_3, X_4, X_1 X_2, X_1 X_3, X_1 X_4, X_2 X_3, X_2 X_4, X_3 X_4, ${X_1}^2$, and X_1 X_2 X_4 were significant terms as their p-values were <0.05. Values greater than 0.1000 indicate that the model terms were not significant. The results also show a correlation between the factors investigated in this study as the interaction of terms was significant. The difference between predicted R^2 (0.9954) and adjusted R^2 (0.9978) was less than 0.2, showing a reasonable agreement. The value of adequate precision (90.431) showed an adequate signal, which suggests that this model can be used to navigate the design space.

Analysis of variance (ANOVA) of the second response, degree of esterification DE% (Table 5), revealed that the model F-value (193.09) infers a significant model. All studied terms were significant except ${X_2}^2$, ${X_3}^2$, and ${X_4}^2$. The results also showed a correlation between the factors investigated in this study as the interaction of terms was significant. The predicted R^2 of 0.9775 was in reasonable agreement with the adjusted R^2 of 0.9883, as the difference was less than 0.2. Adequate precision measures the signal-to-noise ratio, which is a measure of the range in predicted response relative to its associated error. The desired value of adequate precision is four or larger. The value obtained, 45.218, expressed an adequate signal.

Table 4. Analysis of variance (ANOVA) for DSD for pectin yield using ChCl–Mal and its fit statistics.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	
Model	21692.35	12	1807.7	936.46	<0.0001	*significant*
X_1 L/S ratio	902.1	1	902.1	467.32	<0.0001	
X_2Temp	62.17	1	62.17	32.21	<0.0001	
X_3Time	10.04	1	10.04	5.2	0.04	
X_4 NADES/water ratio	154.04	1	154.04	79.8	<0.0001	
X_1 X_2	34.76	1	34.76	18.01	0.001	
X_1 X_3	341.29	1	341.29	176.8	<0.0001	
X_1 X_4	360.69	1	360.69	186.85	<0.0001	
X_2 X_3	102.31	1	102.31	53	<0.0001	
X_2 X_4	57.49	1	57.49	29.78	0.0001	
X_3 X_4	38.04	1	38.04	19.71	0.0007	
${X_1}^2$	236.68	1	236.68	122.61	<0.0001	
${X_2}^2$	0	0				
${X_3}^2$	0	0				
${X_4}^2$	0	0				
X_1 X_2 X_4	15.18	1	15.18	7.86	0.0149	
Lack of fit	15.1757761		1	15.1757761	7.861636586	
Pure error	25.094659		13	1.930358385		

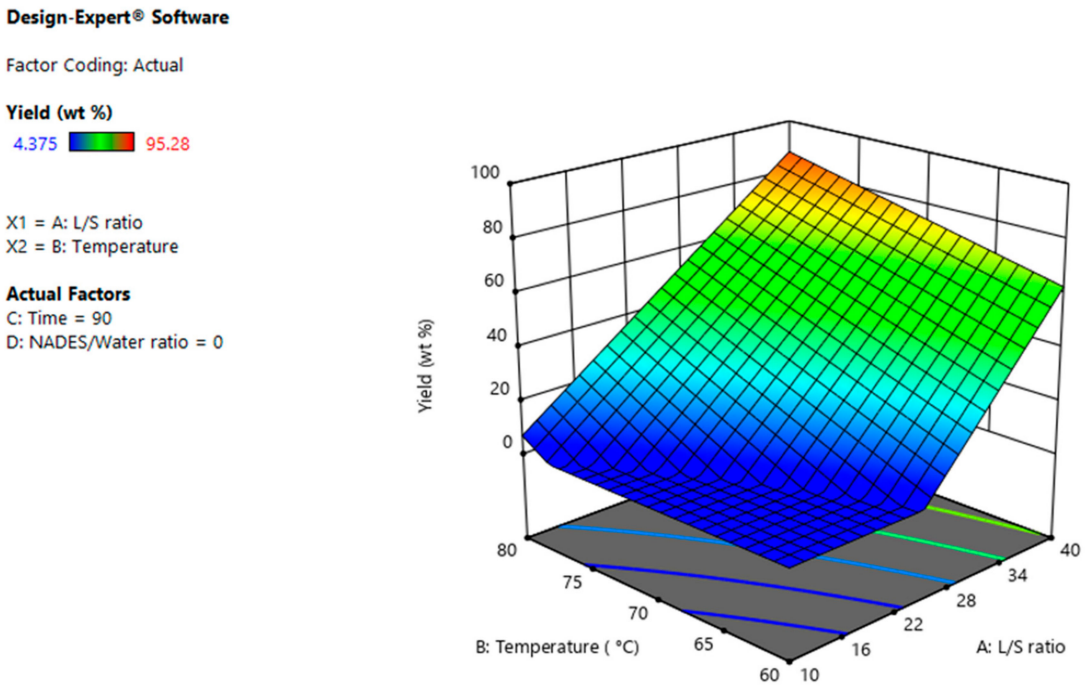
SD	1.39	R^2	0.9988
Mean	33.59	Adjusted R^2	0.9978
C.V.%	4.14	Predicted R^2	0.9954
		Adequate precision	90.4309

Table 5. Analysis of variance (ANOVA) for DSD for DE% using ChCl–Mal and its fit statistics.

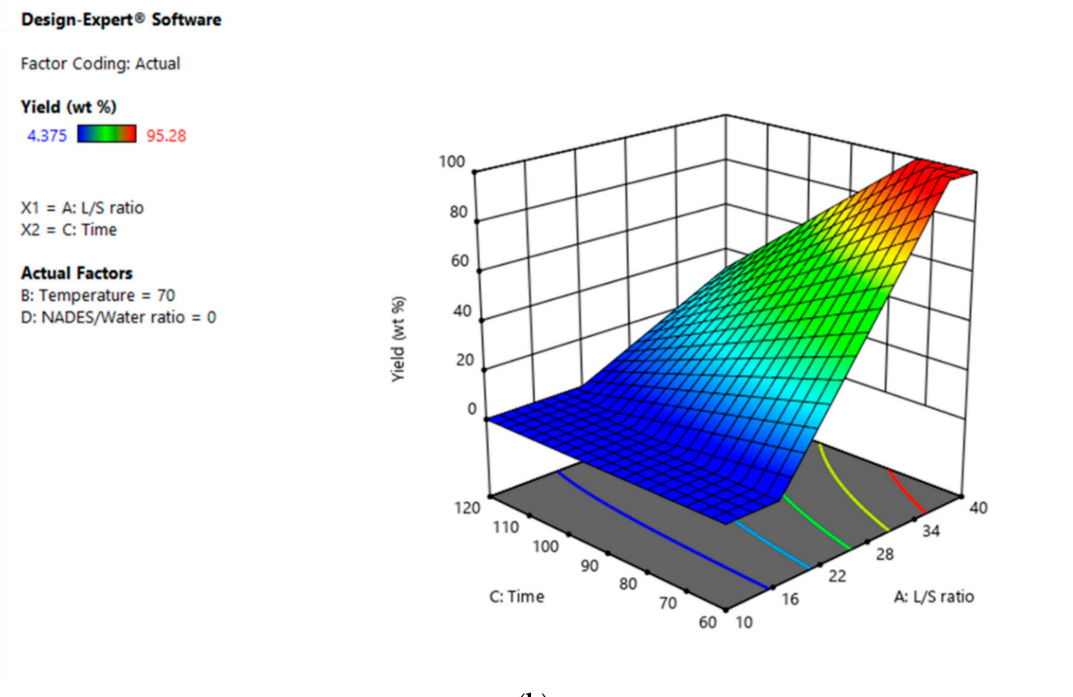
Source	Sum of Squares	df	Mean Square	F-Value	*p*-Value	
Model	1293.74	11	117.61	193.09	<0.0001	*significant*
X_1 L/S ratio	68.47	1	68.47	112.42	<0.0001	
X_2 Temp	42.76	1	42.76	70.20	<0.0001	
X_3 Time	138.10	1	138.10	226.73	<0.0001	
X_4 NADES/Water ratio	222.70	1	222.70	365.61	<0.0001	
X_1 X_2	33.25	1	33.25	54.59	<0.0001	
X_1 X_3	19.28	1	19.28	31.65	<0.0001	
X_1 X_4	55.08	1	55.08	90.43	<0.0001	
X_2 X_3	26.18	1	26.18	42.98	<0.0001	
X_2 X_4	33.71	1	33.71	55.34	<0.0001	
X_3 X_4	88.90	1	88.90	145.95	<0.0001	
X_1^2	31.28	1	31.28	51.35	<0.0001	
X_2^2	0.0000	0				
X_3^2	0.0000	0				
X_4^2	0.0000	0				
Residual	8.53	14	0.6091			
Lack of fit	0.0239	1	0.0239	0.0366	0.8513	*not significant*
Pure error	8.50	13	0.6541			
Cor total	1302.27	25				

SD	0.7805	R^2	0.9935
Mean	52.34	Adjusted R^2	0.9883
C.V.%	1.49	Predicted R^2	0.9775
		Adequate precision	45.2177

The graphs for pectin yield are shown in Figure 1. The response surface shows the optimization region (red). The maximum pectin yield was obtained at 96% with the application of 79 °C, 40 liquid loading, 70 min sonication time, and the maximum amount of NADES/water. This was practically obtained in runs 6 and 10, and agrees with the prediction from the three-dimensional (3D) surface plots.

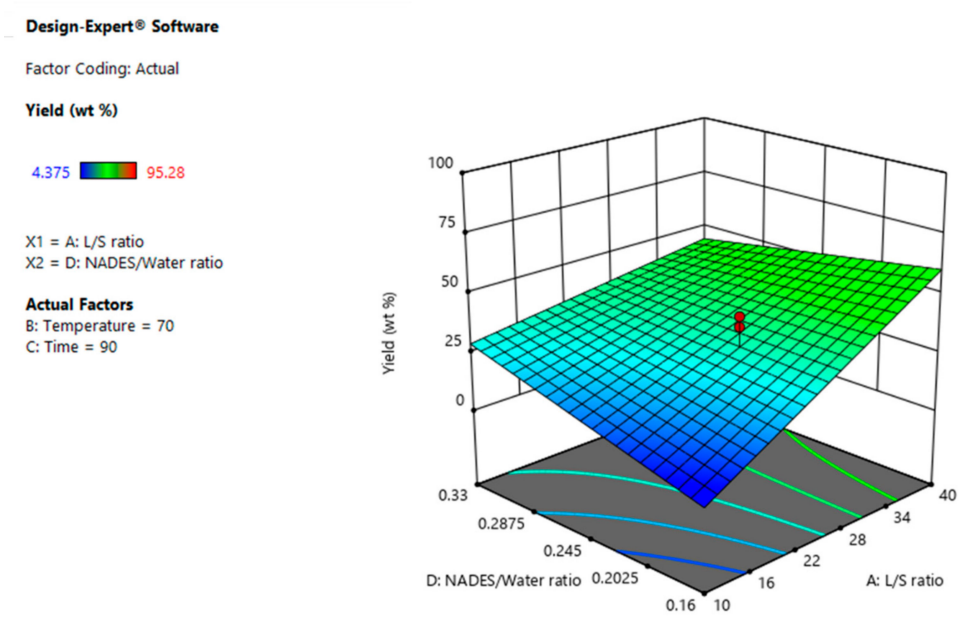


(**a**)

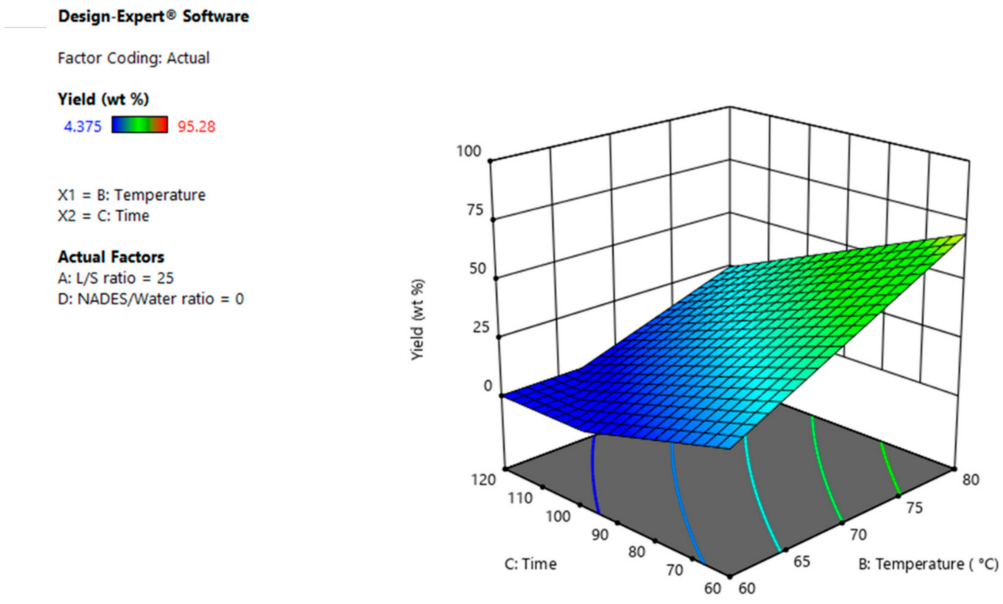


(**b**)

Figure 1. *Cont.*

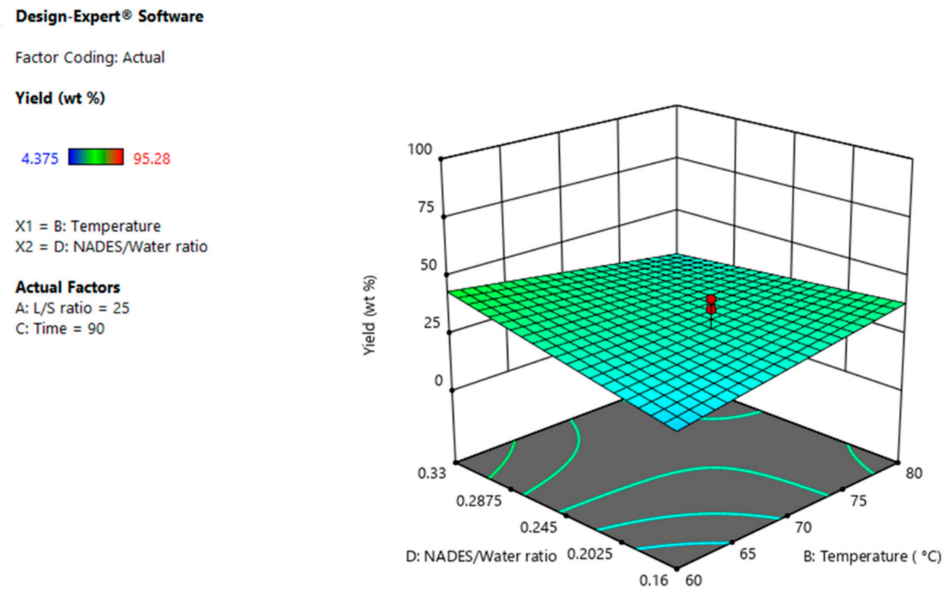


(**c**)

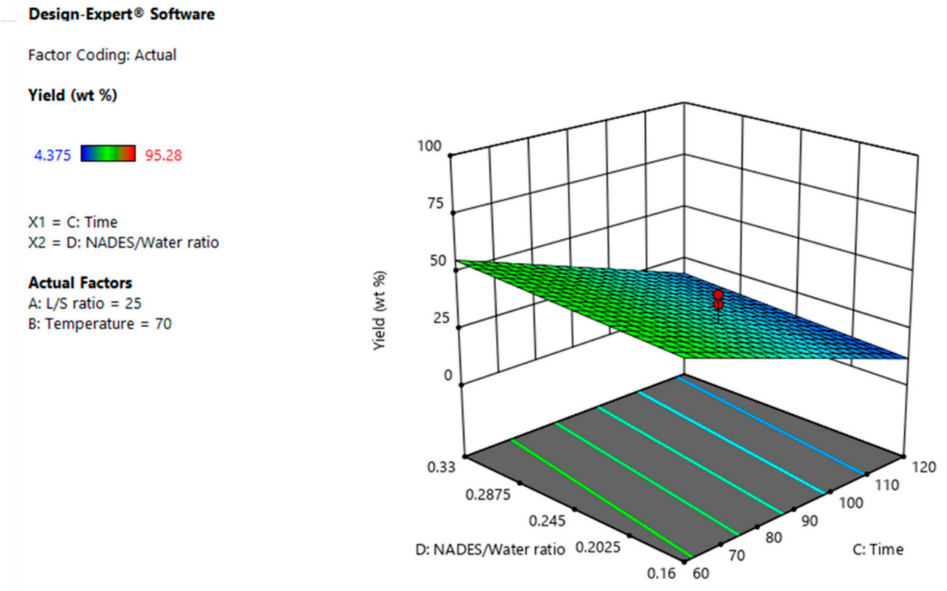


(**d**)

Figure 1. *Cont.*



(**e**)



(**f**)

Figure 1. Three-dimensional (3D) surface plot of pectin yield with four factors (liquid/solid loading, temperature, time, and natural deep eutectic solvent (NADES)/water ratio in ChCl–Mal. (**a**) Liquid/solid ratio vs. temperature; (**b**) liquid/solid ratio vs. time; (**c**) liquid/solid ratio vs. NADES/water ratio; (**d**) temperature vs. time; (**e**) temperature vs. NADES/water ratio; (**f**) time vs. NADES/water ratio.

The degree of esterification DE% ranged from 35–62% with the maximum obtained at runs 6 and 10. It was also observed that the yield was not associated with the DE%, as seen from runs 2 and 9, where high DE% (50–60%) was achieved regardless of the low pectin yield (7–14%).

Validation of the model was conducted based on the solution obtained from the analysis. For instance, one provided solution was liquid/solid ratio 35.23%, temperature 78.2 °C, time 64 min, NADES/water ratio 1:3: 100, desirability = 1, yield = 95.3%, DE = 70.92%. A number of optimization solutions (100) were obtained upon analysis, three of which are presented in Table 6. Three

experiments were performed where experimental and predicted data had a reasonable agreement, which supports the model reliability and the optimal conditions. An error of less than ±10% was recorded. From the optimization, the best conditions occurred at 80 °C, 40:1 liquid/solid ratio, 60 min, and 1:6 NADES/water ratio.

Table 6. Suggested validation parameter for obtaining the maximum pectin yield and DE% during extraction using ChCl–Mal.

No	X_1	X_2	X_2	X_4	Experimental Pectin Yield (wt.%) [a]	Predicted Pectin Yield (wt.%)	Experimental DE% [b]	Predicted DE%	Error %
1	35	78	64	1:3	94.05%	95.28%	66.27%	70.92%	−1.29% [a], −6.56% [b]
2	40	79	60	1:4.5	95.07%	95.64%	60.52%	64.80%	−0.6% [a], −6.60% [b]
3	38	78	63	1:6	96.25%	97.38%	62.47%	69.00%	−1.16% [a], −9.46% [b]

[a] = pectin yield, [b] = degree of esterification DE%.

In the case of ChCl–Glc–W, the ANOVA analysis showed a significant model in the two responses with reasonable R^2 values. Table 7 summarizes the obtained data.

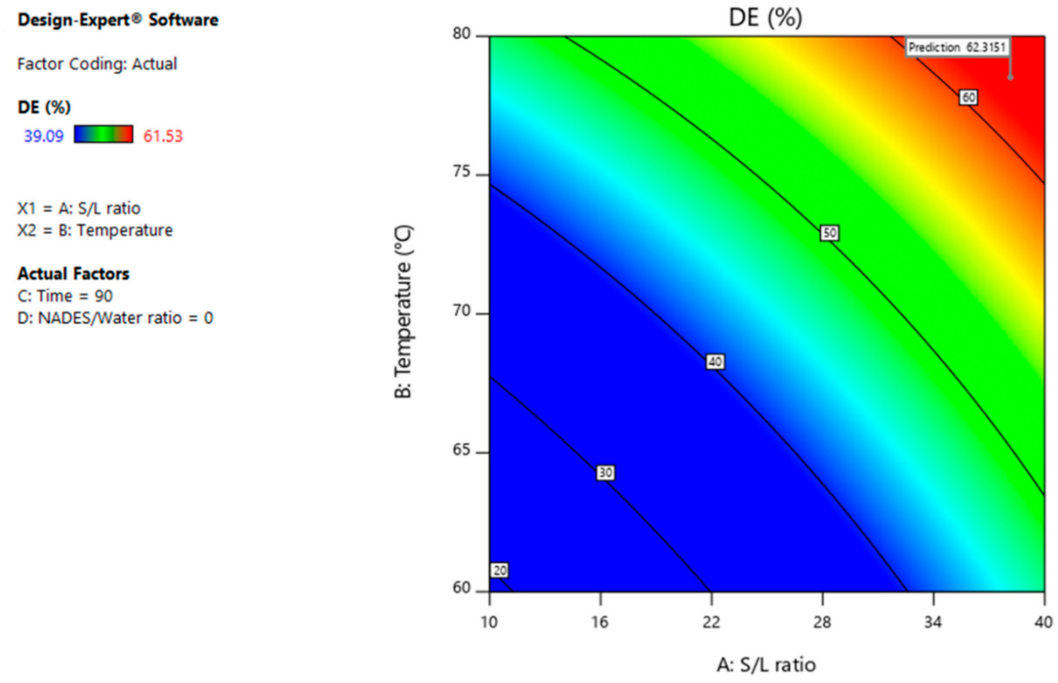
The second response analysis (ANOVA) for DE% (Table 7) revealed that the model F-value (66.54) suggests a significant model. In this study, X_1, X_2, X_3, X_4, X_1X_2, X_1X_4, X_3X_4, and X_1^2 were significant model terms. The results also showed a correlation between the factors investigated in this study as the interaction of terms was significant between liquid/solid ratio and both temperature and NADES/water ratio, and between sonication time and NADES/water ratio. The predicted R^2 0.9345 was in agreement with the adjusted R^2 of 0.9655. The value of adequate precision obtained was 25.63, which shows an adequate signal and endorses the use of the model to navigate the design space.

Table 7. Analysis of variance (ANOVA) for DSD for DE% using ChCl–Glc–W and its fit statistics.

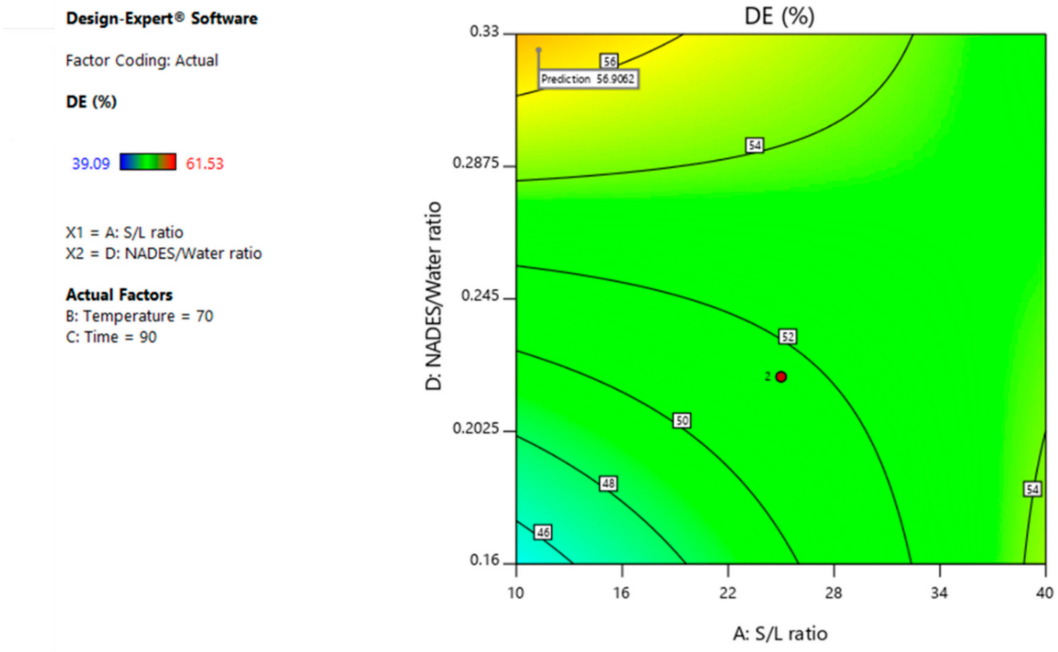
Source	Sum of Squares	df	Mean Square	F-Value	*p*-Value	
Model	1036.33	11	94.21	66.54	<0.0001	*significant*
X_1 S/L ratio	23.93	1	23.93	16.9	0.0011	
X_2 Temp	6.95	1	6.95	4.91	0.0438	
X_3 Time	55.26	1	55.26	39.03	<0.0001	
X_4 NADES/Water ratio	144.78	1	144.78	102.26	<0.0001	
X_1X_2	7.43	1	7.43	5.25	0.038	
X_1X_3	0.5023	1	0.5023	0.3548	0.5609	
X_1X_4	16	1	16	11.3	0.0047	
X_2X_3	0.6233	1	0.6233	0.4402	0.5178	
X_2X_4	3.81	1	3.81	2.69	0.1232	
X_3X_4	27.43	1	27.43	19.37	0.0006	
X_1^2	26.59	1	26.59	18.78	0.0007	
X_2^2	0	0				
X_3^2	0	0				
X_4^2	0	0				
Residual	19.82	14	1.42			
Lack of fit	0.0005	1	0.0005	0.0003	0.9863	*not significant*
Pure error	19.82	13	1.52			
Cor total	1056.15	25				
SD	1.19		R^2		0.9812	
Mean	52.27		Adjusted R^2		0.9665	
C.V.%	2.28		Predicted R^2		0.9345	
			Adequate precision		25.6342	

The model contour maps for pectin DE% response are presented in Figure 2. The response surface shows the optimization region (red). The maximum DE% was predicted in the region of low liquid/solid ratio and very high NADES/water ratio. As predicted, a large amount of NADES should be consumed to achieve the highest DE%, which is not economical. However, the interaction

between the two factors, X_1X_2, showed experimentally similar results to ChCl–Mal. Therefore, it can be observed that both pectin yield and DE% were lower in the case of ChCl–Glc–W, as more NADES was required to achieve the optimum conditions based on the design analysis.

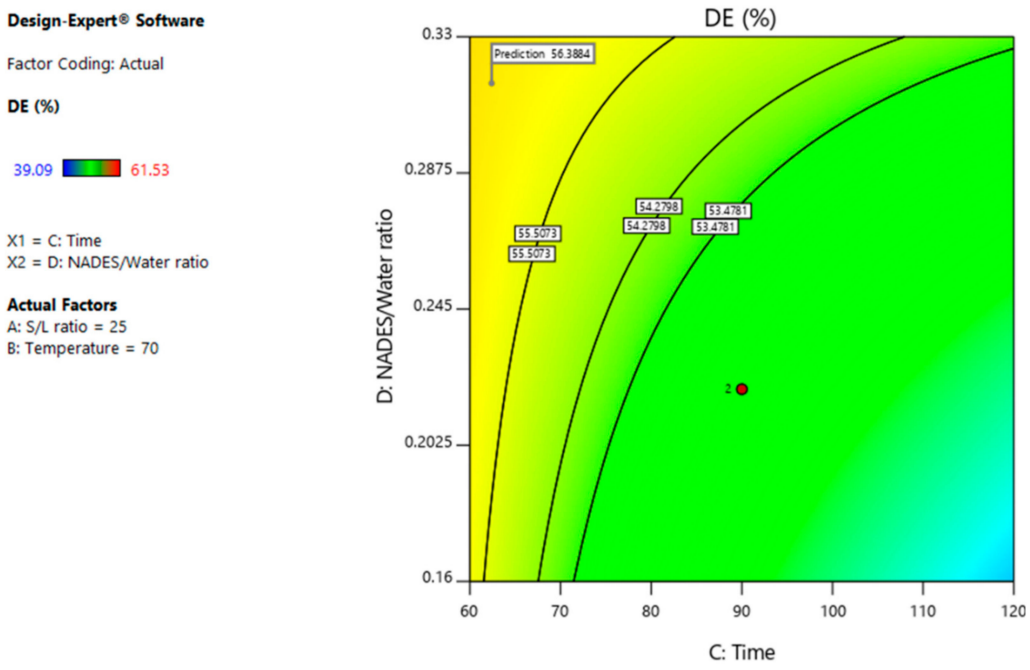


(**a**)



(**b**)

Figure 2. *Cont.*



(**c**)

Figure 2. Contour maps of the pectin DE% with the significant interacting terms in ChCl–Glc–W. (**a**) Liquid/solid ratio vs. temperature; (**b**) liquid/solid ratio vs. NADES/water ratio; (**c**) time vs. NADES/water ratio.

Similarly, validation of the model was conducted based on the solution obtained from the analysis to achieve pectin yield >90 wt.% and DE% higher than 60%. A number of solutions (100) were obtained and three were selected. Three sets of experiments were performed; however, the calculated errors in the case of pectin yield were not in good agreement with predicted data. The provided solutions predicted pectin yield of 98–125%, where the obtained values were in the range of 89–92%. On the other hand, pectin yield did not influence the degree of esterification DE% as they both appeared to respond independently. DE% results of predicted and experimental values were consistent and the recorded errors ranged between 5% and 10%.

From the optimization results, a few observations could be obtained. Firstly, the organic acid-based NADESs are a better choice for pectin extraction compared with other NADESs. This could be possibly attributed to the pH values, as explained earlier in the screening. Moreover, it was reported that organic acids could produce a higher extract than mineral acids [25]. In addition, pectin extraction from citrus fruits displayed a better yield (36.71–67.30%) in the case of citric acid than in inorganic acids such as hydrochloric acid (19.16–21.10%) and nitric acid (19.80–27.63%) [25–27]. It was also distinguished that organic acids require a shorter time for processing if compared with mineral acids as denoted by Liew et al. [2].

Furthermore, as supported by the validation of both ChCl–Mal and ChCl–Glc–W, the organic acid-based NADES favored extraction based on the operating conditions. The predicted and experimental results were reasonable, and no extrapolation was needed. However, it is evidently seen that glucose-based NADES was a second option for pectin extraction regardless of the remarkable pectin yield. It is also a good choice from a green chemistry point of view. To obtain high pectin yield, a longer time was suggested in the case of glucose-based NADES. Liew et al. [2] used DES [lactic acid] [glucose] [water] with a ratio of 6:1:6 for pectin extraction from pomelo powder with the highest pectin yield of 23.04%. However, citric acid had a better yield performance and more energy saving compared to DES, as 39.72% of pectin was obtained.

Abdul Hadi et al. [28] used ChCl–malonic acid (1:1) in the extraction of tocols from crude palm oil (CPO). The DES recorded 18,525 ± 882 mg/kg yield and supported the potential of ChCl–carboxylic acid DES in extraction and separation.

The screening results could also support this, as 120 min of sonication was applied at 60 °C. Likewise, citric acid extraction on pomelo recorded a lower extraction time compared with nitric acid [2], which is desirable in industrial applications.

A study by Raji et al. [17] compared the pectin extraction in several acids (organic and inorganic) at 90 °C and 90 min. They proved that pectin extraction was favorable in carboxylic acids. Citric acid, among eight more tested, was highly recommended for extracting pectin from melon peel with 28.29% yield. In their study, pectin was regarded as low degree, as the range obtained was from 1.33% to 29.33%.

On the other hand, our current study recorded a high degree of esterification, mostly above 50%. It shows that pectin's DE% values were consistent throughout the investigation. The average value of pectin DE% was 52.34%. The highest DE was attained at 1:40 solid/liquid ratio, 78 °C, 1:3 NADES/water ratio, and 60 min of sonication. The results obtained using ChCl–Mal were slightly higher than ChCl–Glc–W regarding pectin yield. The validation of the model gave an average of 95.12% for pectin and an average value of 63.09% for DE, which is in agreement with the prediction. The results convey that the model fits the data obtained experimentally in the case of ChCl–Mal. The galacturonic acid content of good-quality pectin determined by the Food and Agriculture Organization (FAO) is above 65% [26]. Therefore, pomelo is a potential source of HM pectin.

3.3. Observation of Pectin Sample Morphology

The micrographs of pectin obtained from ChCl–Mal and ChCl–Glc–W were compared after filtration and drying using FESEM images, as shown in Figure 3.

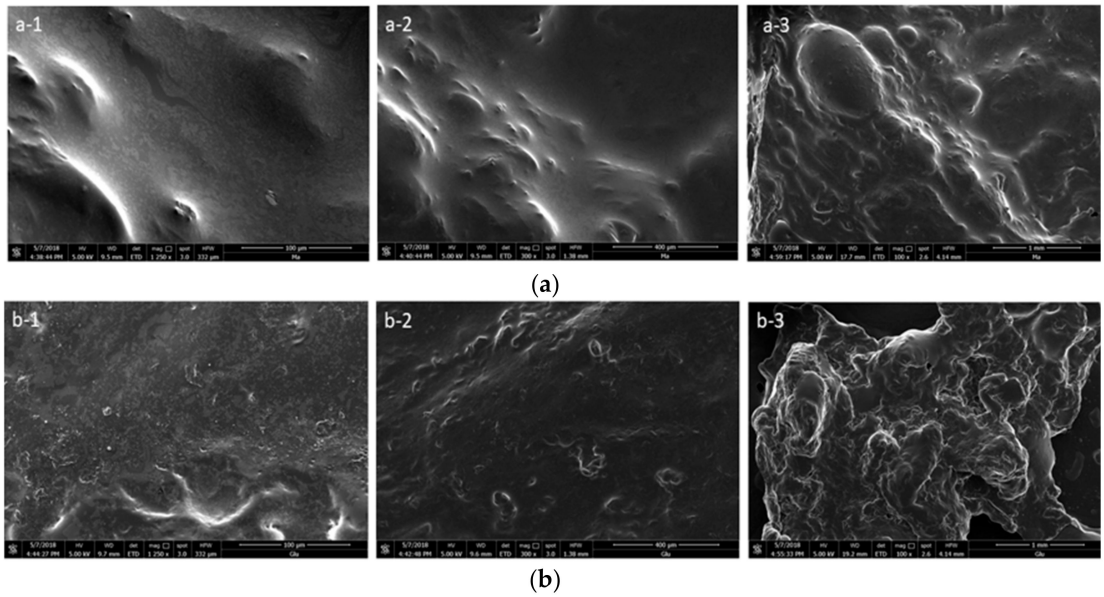
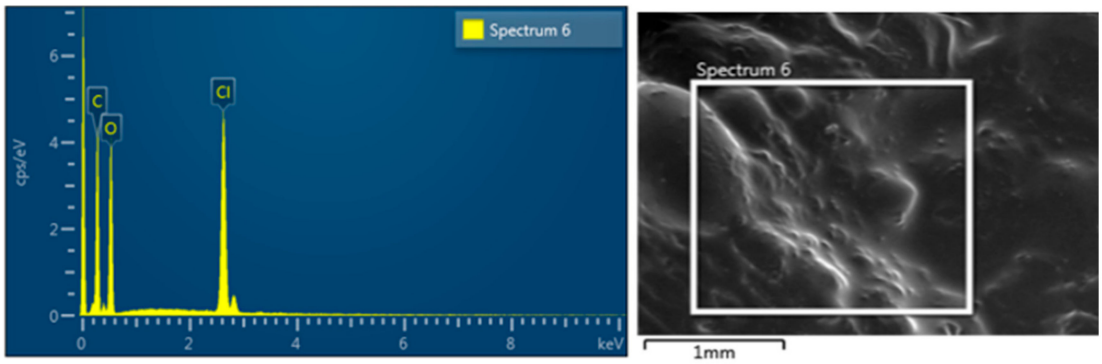


Figure 3. (**a**) Electron images of pectin extracted by ChCl–Mal; a-1 (1250×), a-2 (300×) a-3 (100×). (**b**) Electron images of pectin extracted by ChCl–Glc–W; b-1 (1250×), b-2 (300×) b-3 (100×).

The EDS [29] data of Figure 4a,b shows that the weight ratio of carbon decreased slightly in the case of malonic acid NADES while Glc provided a higher ratio of carbon (56.17%). It is predicted that, due to the presence of glucose in the NADES, part of it was not washed out and condensed in the system during heating and sonication. The spectra showed approximately similar weight ratios for the elements C, O, and Cl. The presence of Cl is attributed to choline chloride from the NADES.

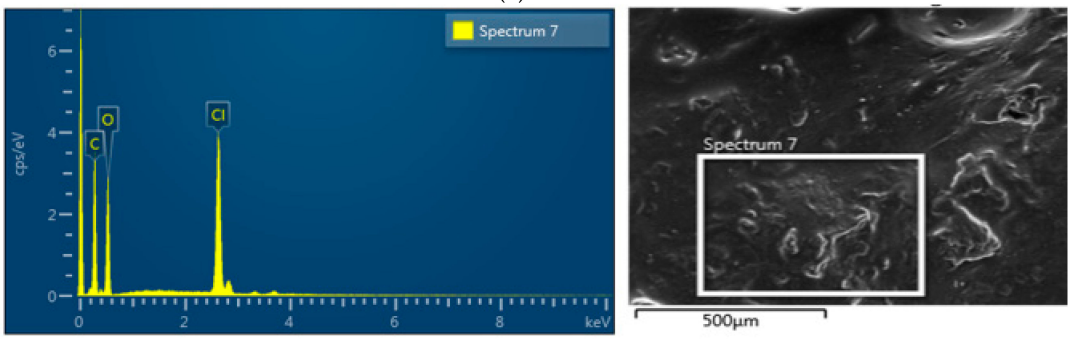
The examination of the structures of both acid- and glucose-extracted pectin revealed a few distinctive features. While acid-based pectin appears smooth, metallic, and shiny, glucose-based pectin appears rough. The images showed the compact and attached structure of the acid pectin (Figure 3).

The obtained electron images also agreed with the digital images taken for both samples in Figure 5. The acid-based pectin was easy to filter and came as a compact and gelatinous structure, while the glucose-based pectin was harder to filter and had a loose construction.



Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma
C	K series	16.41	0.16413	54.42	0.45
O	K series	23.01	0.07744	35.86	0.42
Cl	K series	12.60	0.11008	9.72	0.13
Total:				100.00	

(**a**)



Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma
C	K series	13.09	0.13091	56.17	0.50
O	K series	16.52	0.05558	33.53	0.47
Cl	K series	10.68	0.09330	10.31	0.15
Total:				100.00	

(**b**)

Figure 4. (**a**) Energy-dispersive X-ray spectroscopy (EDS) spectrum of pectin extracted by ChCl–Mal and its electron image with element composition; (**b**) EDS spectrum of pectin extracted by ChCl–Glc–W and its electron image with element composition.

The use of NADES for the isolation of pectin from pomelo peels holds great promise. A number of NADESs showed an extraction yield of pectin 2–11 times higher compared to the 0.05 M HCl that is used in the industry. The extraction process using NADES would differ only during the precipitation

step. Currently, using the HCl extract, the liquid is dried down before adding a set volume of ethanol, which decreases the amount required for the precipitation step. When using NADES, drying down the liquid extract is not an option. Thus, a more significant amount of ethanol is needed for the precipitation. However, evaporation of ethanol from NADES is easy, which enables recycling the ethanol. Moreover, developing a process to recycle and reuse the NADES will be essential for making this process economically feasible [20]. The pectin obtained in this study requires more purification to be used in food application. Nevertheless, the process using NADES is promising with high yield and good quality of pectin.

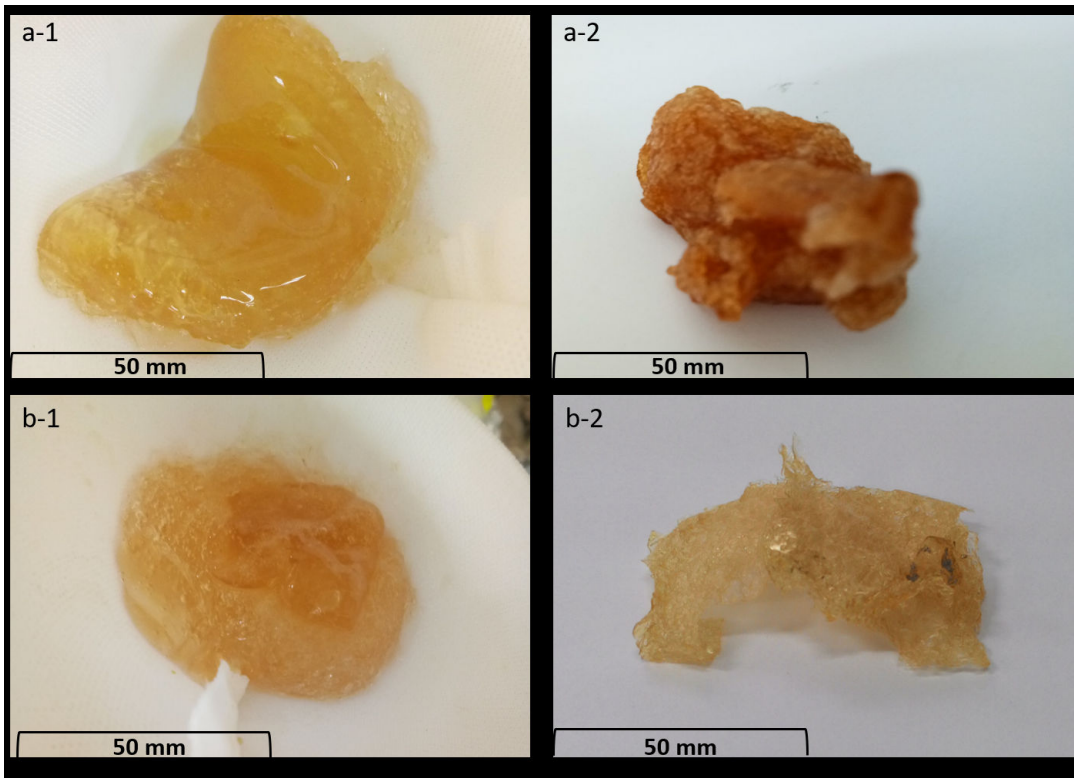


Figure 5. (**a**) Digital images of pectin extracted by ChCl–Mal; a-1 (after filtration), a-2 (after drying). (**b**) Digital images of pectin extracted by ChCl–Glc–W; b-1 (after filtration), b-2 (after drying).

4. Conclusions

The pectin extraction from pomelo peel using choline chloride–malonic acid showed a high yield and an average degree of esterification of 52% at 80 °C, for 60 min at a 40:1 liquid/solid ratio. The developed second-order models from the optimization study predicted both yield and DE% values accurately in the case of ChCl–Mal, with adequate determination coefficients. The morphology of the pectin structure also showed the smooth structure of pectin extracted by ChCl–Mal compared to glucose-based NADES. Comparatively, acid-based pectin extraction is more efficient than sugar-based NADES in terms of its yield and structure. Developing a recycling pathway is required for this approach to be applied at an industrial scale.

Author Contributions: The conceptualization of the research was done by A.H. and M.H.; the methodology was outlined by M.E.S.M., G.C.N., and S.Q.L.; the design of the experiment and its execution were performed by A.A.E.; validation of the model was done by M.Y.Z.b.M.Y. and Y.A.; formal analysis and discussion of the results were done by A.A.E.; investigation and laboratory work were performed by A.A.E. and S.N.R.; resources were provided by M.R.M.N. and Y.A.; data curation, A.A.E.; writing—original draft preparation was done by A.A.E.; writing—review and editing was done by H.M.S.; visualization was done by A.A.E. and A.H.; the project was supervised by A.H. and M.H.; project administration, A.H. and S.N.R.; funding acquisition, M.R.M.N., Y.A., and H.M.S.

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Conflicts of Interest: The authors declare no conflicts of interest.

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