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ORIGINAL PAPER

Multi-objective optimization of pectin extraction from orange peel via response surface methodology: yield and degree of esterification

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Abstract

Pectin is extensively extracted from citrus peel for applications in food, nutrition, cosmetics and pharmaceutical industries. In this study, extraction of pectin from orange peels was attempted using different types of acid solvent, namely acetic, hydrochloric, nitric and citric acids. Fourier Transform Infrared spectroscopy revealed that distinctive functional groups of pectin were found in the extracted pectin samples using all acid solvents and the spectra were comparable to the commercial pectin reported in literature. Citric acid was identified as the best extracting solvent for the highest pectin yield (3.82%) and optimum degree of esterification (DE) (> 50%) for the extracted pectin. Multi-objective optimization of the extraction process was performed using Response Surface Methodology on the yield and degree of esterification. A five-level Central Composite Design was adopted for the experimental design involving three process variables namely extraction temperature (60–90 °C), solvent pH (2–4) and extraction time (30–90 min). The highest pectin yield at 35.20 ± 0.39% and DE of 45.43 ± 0.39% was achieved at the suggested optimum extraction conditions at 90 °C, solvent pH 2.19 and 66 min. Verification of the optimization showed that the percentage error difference between the experimental and predicted results was less than 10%, indicating significance of the established extraction model and correlation. Interestingly, the DE (< 50%) of the extracted pectin at optimal conditions revealed that low methoxyl (LM) pectin was obtained rather than high methoxyl (HM) pectin as extracted during the solvent screening process.

Keywords Pectin · Multi-objective optimization · Extraction · Yield · Degree of esterification

Introduction

In 2020, the Food and Agriculture Organization of the United Nations estimated that the worldwide production of citrus fruits in year 2018 was approximately 152 million tonnes [14]. Based on a recent report by Mahato et al. [34], about 110 million tonnes of citrus fruit wastes are generated from the fruit processing industries throughout the world annually. The non-edible parts of citrus fruits such as peels and seeds are generally discarded as wastes with the peels accounted for almost 50 to 70% of the total processed citrus fruits [64]. These wastes are mostly disposed of in landfill which lead to many environmental problems if they are not treated appropriately and adequately prior to landfill disposal. This means that bioactive components present in the peels and seeds such as sugar, carbohydrate, protein, fats, organic acids, flavonoids, oil and pigments [2] are discarded as wastes from the fruit processing industries. Therefore, numerous efforts have been demonstrated to minimize the citrus wastes disposal into the environment and convert them into value-added products. Orange is a popular citrus fruit all over the world with annual production of 75 million tonnes globally [14]. Similar to other citrus fruits, orange waste is composed of 60 to 65% of peels and 30 to 35% of pulp and seeds [54]. Orange peel waste (OPW) contains ash (2.46%), fat (3.85%), flavonoids (4%), lignin (6.93%), protein (9%), soluble sugars (9%), hemicellulose (9.99%), pectin (20.9%)

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and cellulose (33.98%) [47]. Generally, the high pectin content suggests OPW as a potential source of pectin extraction, which is currently in high demand for pharmaceutical and food applications.

Pectin is a naturally occurring polysaccharide in the intracellular structure and cell wall of plants [15] which consists of three main structural components, namely the homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II [42]. Pectin consists of a long chain of galacturonic acid units that are linked via α-1,4 glycosidic bonds. The galacturonic acid chain acts as the homogalacturonan backbone of pectin which some neutral sugars such as rhamnose, xylose, galactose and arabinose are attached to it as side chains [27]. Esterification of pectin occurs when the galacturonic acid chain is partially esterified to form methyl ester and acetyl ester [15]. The percentage of the galacturonic acid units that are esterified out of the total galacturonic acid units in the chain is referred to as degree of esterification (DE) [38]. DE is a significant property that differentiates the types of pectin and affects its gelling ability. As mentioned by Liu et al. [31], pectin with DE value higher than 50% is known as high methoxyl (HM) pectin whereas the pectin is categorised as low methoxyl (LM) if the DE value is lower than 50%.

Pectin is commonly utilized in food applications such as the production of jam and jelly due to its gelling properties [58]. In particular, the difference in DE values indicates the suitability of pectin for some specific applications. For example, HM pectin is usually used as gelling agent in the manufacturing of high sugar products while LM pectin is used in the manufacturing of low sugar products [8]. Apart from food applications, pectin is utilized in the pharmaceutical application to lower cholesterol level in human body [3]. Also, it is reported as drug carrier for colon treatment in medical industry [32]. Recently, the applications of pectin have diversified into food packaging and it is extensively studied as an alternative environmental friendly material to replace synthetic plastics [51] due to biodegradability and the food preservation properties that are similar to the synthetic plastics [12]. In view of increasing demand for pectin in various applications, its extraction from citrus peels particularly orange peels is deemed interesting to obtain this biomaterial from waste resources.

Pectin is commonly extracted with hot dilute acidified solution, followed by the precipitation process with the addition of alcohol [1]. Mineral acids such as sulfuric acid, hydrochloric acid and nitric acid are commonly used as the extracting solvent [43] due to lower cost and possibility to produce pectin with enriched homogalacturonan blocks [41]. Nevertheless, the usage of mineral acids has been called into question due to their toxicity and impacts to the environment [44]. Consequently, weak organic acids such as citric acid [40, 43] and tartaric acid [36, 55] have also been applied in the recent studies on pectin extraction to countermeasure the downsides of mineral acids. A few previous works revealed that the yield or characteristics of extracted pectin could be affected by the types of acid ([59, 29] and extraction conditions [17, 48, 50]. To date, the comparison of the efficiency of both organic and inorganic acids to extract pectin remains limited in the literature. Majority of the reports are associated with the discovery of alternative sources for pectin extraction. To the authors' best knowledge, multi-objective optimization of the pectin extraction process considering both the pectin yield and quality in terms of DE value is also yet to be reported. Acknowledging the fact that DE values are correlated to the different possible applications of pectin, it is important to identify the relationship between the yield and quality of the extracted pectin under the influence of various extraction parameters.

The present study aims to investigate the yield and DE value of pectin extracted from orange peels using both organic acid (acetic and citric acids) and inorganic acid (hydrochloric and nitric acid), and analyse the structural characteristic of all extracted pectin using Fourier Transform Infrared Spectroscopy (FTIR) scanning. Subsequently, multi-objective optimization of the pectin extraction process based on extraction parameters such as extraction temperature, solution pH and extraction duration with respect to both pectin yield and DE is performed.

Materials and method

Chemicals and raw materials

Orange fruits were purchased from a wet market nearby UCSI University, Kuala Lumpur, Malaysia. Unless otherwise specified, hydrochloric acid (Merck), nitric acid (Merck), citric acid (Merck), acetic acid (Merck), ethanol (R&M Chemicals), sodium hydroxide (R&M Chemicals) and phenolphthalein (Merck) were used as received without further purification.

Preparation of orange peels

Orange peels were separated from the fruits, washed and cut into small pieces using a knife. Thereafter, the orange peel pieces were dried in oven at 60˚C until a constant weight was attained. The dried peels were then subjected to a blender to reduce its particle size and were sieved through a strainer to obtain powder with uniform size. The dried orange peel powder was packed in airtight sealable bag and stored in the fridge prior to the pectin extraction process.

Extraction of pectin from orange peels

The pectin extraction was performed according to the procedures described by Pasandide et al. [41] and Canteri-Schemin et al. [4]. First, pH of distilled water was adjusted to 3 using acid solvent to obtain acidified distilled water. 5 g of the dried orange peel powder was then mixed with the acidified distilled water at solid to liquid ratio of 1:30 (w/*v*). The mixture was heated at 60 °C for 1 h using a magnetic hotplate stirrer with continuous stirring. After that, the hot acid extract was cooled to room temperature and filtered through a filter paper to obtain the filtrate that consisted of the soluble pectin [1]. A double volume of 95% ethanol was added into the filtrate and the mixture was stored at room temperature for 24 h to allow for precipitation of pectin. After 24 h, the pectin formed was retrieved by filtering the mixture through a filter paper. The wet pectin collected was washed with 70% ethanol and dried in an oven until the weight was unchanged. The yield of pectin was calculated based on Eq. (1)

$$\text{Yield}(\%) = \frac{\text{Mass of extracted pectin (g)}}{\text{Mass of dried orange peel powder (g)}} \times 100\% \quad (1)$$

Comparison of extracting acid solvent

The aforementioned pectin extraction procedures were repeated to extract pectin from orange peel powder with 0.1 M of organic acids (acetic acid and citric acid) and inorganic acids (hydrochloric acid and nitric acid), respectively. The yield and DE of the orange peel pectin extracted using different acid solvents were compared and the best extracting solvent was selected for the subsequent optimization study in this study.

Characterization of orange peel pectin

Functional group analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to identify the structural characteristics of the pectin extracted from orange peel powder using different acid extracting solvents (nitric acid, acetic acid, hydrochloric acid and citric acid). The spectra were obtained using a FTIR Spectrometer (PerkinElmer Spectrum Two) in the range 4000 to 500 cm^{-1}.

Degree of esterification (DE)

The DE of pectin was measured using titration method described by Pasandide et al. [41]. First, the extracted pectin was moistened with an adequate amount of 95% ethanol to facilitate its dispersion in the water. The moistened pectin was dissolved in distilled water at solid to liquid ratio of 1:200 (w/*v*). The mixture was stirred using a magnetic hotplate stirrer at 40 °C until the pectin was fully dissolved in the water. Subsequently, two drops of phenolphthalein were added into the solution and it was titrated using 0.25 M sodium hydroxide solution to neutralize the free carboxyl acid groups from the polygalacturonic acid [30]. The volume of sodium hydroxide solution used to turn the dissolved pectin solution from colourless to pink colour was recorded as V_1.Then, 10 ml of 0.25 M sodium hydroxide was added into the solution and stirred for 30 min in order to allow for hydrolysis process to take place [53]. Subsequently, 10 ml of 0.25 M hydrochloric acid solution was added into the solution to neutralize the sodium hydroxide and the solution was stirred until its colour changed from pink to colourless. Two drops of phenolphthalein were added into the solution again and the solution was re-titrated with 0.25 M sodium hydroxide until colour changed from colourless to pink. The volume of sodium hydroxide solution required for the second titration was recorded as V_2. The DE of pectin was calculated based on Eq. (2).

$$\text{DE}(\%) = \frac{V_2(\text{ml})}{V_1(\text{ml}) + V_2(\text{ml})} \times 100\% \quad (2)$$

Multi-objective optimization pectin extraction process

Design of experiment

In this work, a five-level Central Composite Design (CCD) in Design Expert version 11.0 was applied to develop the experimental design for optimization of pectin extraction process. The independent process variables that were investigated included extraction temperature (A), solvent pH (B) and extraction time (C) while the response variables recorded were the pectin yield (%) and DE value (%), respectively. The range of the process variables studied in this study is tabulated in Table 1. In order to evaluate the effects of extraction process variables, 20 experiments were performed in random to cover all combinations of the factor levels in the CCD design.

Statistical analysis and validation of optimum conditions

Statistical analysis of the experimental results was performed in the form of analysis of variance (ANoVA) by using the Design Expert software. The analysis included the F-test associated with probability p(F) and determination of the coefficient, R^2. The adequacy of the generated models was also validated by carrying out pectin extraction process

Table 1 Independent process variables and their respective levels in experimental design

Variables	Unit	Range	Symbol	Level				
				$-\alpha$	−1	0	+1	$+\alpha$
Extraction temperature	°C	60–90	*A*	49.77	60.0	75.0	90.0	100.23
Solvent pH	–	2–4	*B*	1.32	2.0	3.0	4.0	4.68
Extraction time	mins	30–90	*C*	9.55	30.0	60.0	90.0	110.45

Table 2 Yield and DE of pectin extracted from orange peels using different acids

Type of acid	Acid solvent	Yield (%)	DE (%)
Organic	Acetic acid	1.95	75
	Citric acid	3.82	59
Inorganic	Hydrochloric acid	1.57	60
	Nitric acid	3.82	55

at the predicted optimized conditions in triplicate and subsequently the average value of the experimental pectin yield and DE value were compared with the predicted results.

Results and discussion

Effects of different extracting acid solvents

The applicability of four different acid solvents (acetic acid, citric acid, hydrochloric acid, and nitric acid) in pectin extraction from orange peels was evaluated prior to the optimization of extraction conditions. The yield and DE results of pectin extracted from orange peels using different acid solvents are shown in Table 2.

The extraction results showed that the highest pectin yield was obtained when citric acid and nitric acid were used, followed by acetic acid and the lowest pectin yield was resulted by hydrochloric acid. The findings were consistent with a number of previous studies in which different amount of pectin was extracted when different types of acid solvent used in the extraction process, with citric acid yielded the best result. To name a few, Lee and Choo [28] reported that higher pectin yield was extracted from watermelon rind at optimum conditions when citric acid ($8.38 \pm 0.43\%$) was used as compared to the hydrochloric acid ($6.52 \pm 0.15\%$). Besides, the superiority of citric acid (56.6%) over hydrochloric acid (12%) and sulfuric acid (12.6%) was reported by Gazala et al. [16] who extracted pectin from apple pomace. Also, Chan and Choo [5] reported that the highest range of pectin yield was obtained from cocoa husk using citric acid (3.58–7.62%) compared to hydrochloric acid (3.62–6.01%). These results resonate with the findings of Canteri-Schemin et al. [4] who studied on the effect of different acids (hydrochloric, citric, nitric, sulfuric, phosphoric, tartaric, and malic acid) on pectin yield and reported that the highest average yield (13.75%) was extracted from apple pomace using citric acid.

Based on the results in Table 2, it was found that organic and inorganic acid solvents showed comparable performance in extracting pectin from orange peels. The difference in the yield could be attributed to the difference of strength between the acid solvents investigated. A strong acid such as hydrochloric acid tends to cause partial hydrolysis reaction to happen more easily, hence resulted in smaller pectin molecules with higher solubility [25, 26]. Consequently, precipitation of pectin molecules was impeded despite the addition of alcohol. For inorganic acid, it was found that acetic acid resulted in a higher pectin yield compared to hydrochloric acid which could be associated with the above-mentioned reason as hydrochloric acid is generally known as a strong acid whereas acetic acid is a weak acid. On the other hand, while nitric and hydrochloric acids are both categorized as strong acids, higher pectin yield was obtained when nitric acid was used as the extracting solvent compared to hydrochloric acid. This could probably because nitric acid has a lower strength compared to the latter, thus not promoting significant partial hydrolysis reaction to occur. The degradation of pectin into smaller molecules was therefore lower.

Apart from pectin yield, the effect of different acid solvents on the DE of extracted orange peel pectin was also investigated. It was obvious that high level of DE (> 50%) was obtained for all extracted pectin regardless of the type of acid solvents, indicated that the pectin was of HM grade. As reported in the literature, HM pectin could also be extracted from other sources such as grapefruit peel with DE ranged from 70.73 to 75.53% [53], apple pomace with optimum DE at 83.20% [11], coffee pulp with DE of 93.75% [46], and passion fruit peel with DE of 60.36% [40]. The present study found that the DE of pectin extracted from orange peels was affected by the type of acid solvents used. All the extracted pectin demonstrated different DE values whereby the highest DE was obtained when acetic acid was used as the extracting solvent, followed by hydrochloric acid and citric acid, and the pectin extracted using nitric acid showed the lowest DE. The trend was similar to the work of Luo et al. [33] with the highest ($82.66 \pm 0.46\%$) and lowest ($70.40 \pm 0.86\%$) DE were obtained by using acetic acid and nitric acid, respectively for the pectin extraction from apple pomace among other solvents (hydrochloric and sulfuric acid). From Table 2, it was

also evident that there was no significant difference between the DE of pectin extracted using hydrochloric acid and citric acid. However, Jiang et al. [24] reported that pectin with higher DE was extracted from *Akebia trifoliata* var. *australis* peel with citric acid (76.64%) compared to hydrochloric acid (59.46%) thereby indicated citric acid caused a lower de-esterification effect on pectin. According to Yapo [61], citric acid caused the least de-esterifying effect as compared to the mineral acids during extraction process from yellow passion fruit rind, hence the extracted pectin demonstrated the highest DE as compared to sulfuric and nitric acids. The discrepancy between the results in this study with literature could be due to the use of different source of pectin and extraction conditions.

Based on the extraction results using different acid solvents, citric acid was identified as the most suitable solvent to be used for the subsequent optimization study in this work. Apart from the highest pectin yield and optimum DE value (within the range of HM) of the extracted orange peel pectin, it was also more environmental friendly to select organic acid such as citric acid compared to the mineral acids, to be the extracting solvent [40, 43].

Functional group analysis

FTIR analysis was carried out to identify the main functional groups of pectin extracted from orange peels using different acid extracting solvents, thus validating the presence of the pectin. The FTIR spectra obtained for pectin samples extracted by using different acid (acetic acid, nitric acid, hydrochloric acid, and citric acid) extracting solvents are illustrated in Fig. 1. Generally, similar FTIR spectrum was observed among all pectin samples despite different extracting solvents were used.

Based on the FTIR spectra, a peak could be observed at a broad range between 3000 and 3700 cm^{-1} which was owed to the presence of OH functional groups in the pectin molecular structure. This region indicated the presence of inter- and intramolecular hydrogen bond in the galacturonic acid structure of the pectin molecule [19, 52]. Another peak found at the region between 2800 and 3000 cm^{-1} was ascribed to the presence of CH group. At the region between 1680 and 1810 cm^{-1}, an absorption peak was detected due to the stretching vibration of carbonyl functional groups (C=O) of methyl esterified carboxylic groups [18, 39]. In addition, a peak at wavenumber ranged between 1490 and 1700 cm^{-1} represented the stretching vibration of C=O group of carboxylate functional groups (COO–) in the pectin structure [40].

The absorption band between 800 and 1200 cm^{-1} represented the unique characteristic of the pectin molecule which this region was known as "fingerprint" region and it could be hardly interpreted based on the functional groups [56]. In general, the fingerprint region comprises a complex absorption pattern which is used to identify a particular compound. According to Lee and Choo [28], the vibration of pyranose cycle could be detected at the pectin fingerprint region between 950 and 1200 cm^{-1}. Referring to the functional groups of commercial pectin corresponding to the FTIR wavenumber reported by Raji et al. [45], comparison was made on the significant functional groups between orange peel pectin extracted in this study using different acid extracting solvents and the commercial pectin. As shown in Table 3, the FTIR wavenumber obtained from orange peel pectin was similar to the spectra wavenumber of commercial pectin. This reveals that characteristics of orange peels pectin extracted in this study were comparable to the commercial pectin.

Multi-objective optimization of pectin extraction process

In this study, RSM with the experimental design of five-level CCD was applied to optimize the pectin extraction process using citric acid. A total of 20 experimental runs were carried out at different extraction conditions which the predicted and experimental results with respect to each run are tabulated in Table 4. The experimental result was subjected to regression and ANoVA analysis using Stat-Ease Design Expert version 11.0 software. Regression analysis was performed to generate a quadratic order polynomial model which the model was subsequently fitted to the experimental responses to describe the relationship between the independent variables (extraction temperature, solvent pH, and extraction time) and the responses (pectin yield and DE). The quadratic order polynomial model for pectin yield and DE was expressed in Eqs. (1) and (2), respectively.

$$\begin{aligned}\text{Yield }(\%) =& 72.72999 + 0.452817A - 58.31178B + 0.621376C - 0.182000AB - 0.004989AC \\ & - 0.108417BC + 0.003960A^2 + 10.17177B^2 + 0.001320C^2\end{aligned} \quad (3)$$

$$\begin{aligned}\text{DE }(\%) =& - 305.44364 + 3.90801A + 135.05406B + 0.394567C - 0.383000AB + 0.002878AC \\ & - 0.029250BC - 0.020731A^2 - 13.41665B^2 - 0.005342C^2\end{aligned} \quad (4)$$

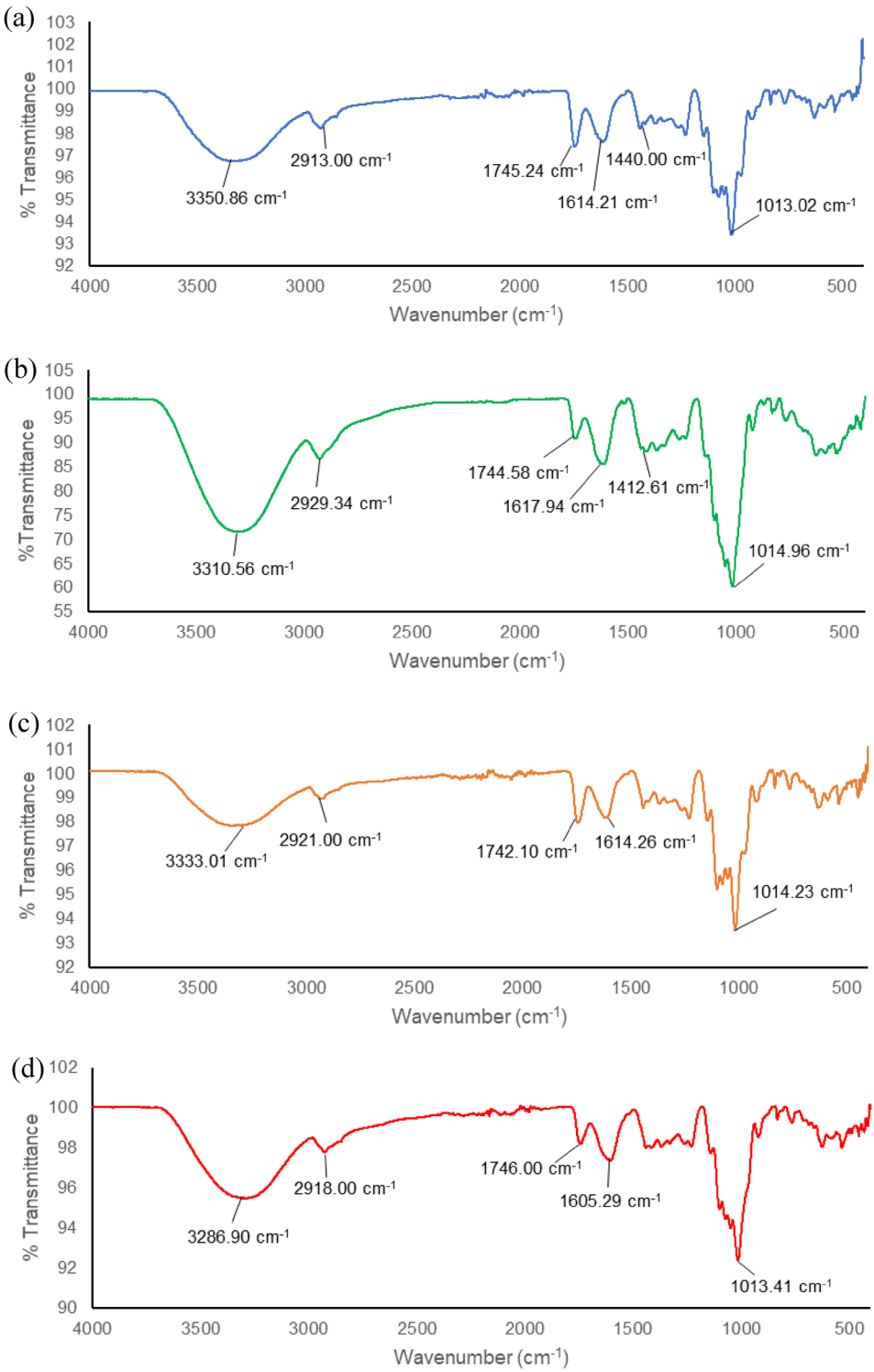

Fig. 1 FTIR spectra of orange peel pectin extracted using different acid extracting solvents: **a** acetic acid, **b** nitric acid, **c** hydrochloric acid; and **d** citric acid

The quadratic models represented the relationship between the independent variables and responses whereby A, B and C were the coded term corresponding to the variables of extraction temperature, solvent pH and extraction time, respectively. The effect of independent variables on the responses could be predicted by the sign of the regression coefficient of the models. Positive sign of regression coefficient indicated the associated variable could lead to a positive effect on the response while negative sign could lead to an opposite effect on the response [62]. For

Table 3 FTIR spectra and peak assignment of orange peels pectin extracted using different acid extracting solvents (this study) and commercial pectin [45]

FTIR wavenumber (cm^{-1})					Functional Group
A	B	C	D	E	
3351	3311	3333	3287	3389	OH group
2913	2929	2921	2918	2940	CH group
1745	1745	1742	1746	1753	C=O from methyl esterified carboxylic group
1440 and 1614	1413 and 1618	1437 and 1614	1439 and 1605	1441 and 1630	C=O from carboxylate group
800–1200	800–1200	800–1200	800–1200	800–1200	Fingerprint

Orange peel pectin extracted by *A* acetic acid, *B* nitric acid, *C* hydrochloric acid, *D* citric acid, *E* commercial pectin

instance, the positive sign associated with the linear term A showed in Eq. (1) represented that increasing of A could increase the pectin yield and the negative sign associated with the linear term B represented that an increase in B could lead to a decrease of pectin yield.

The result of ANoVA and regression analysis were tabulated in Table 5. As presented in the result, the *F*-value of the model for yield and DE was 27.57 and 6.40 respectively, while the *p*-value was less than 0.05 for both models. The high *F*-value and low *p*-value (<0.05) has validated that the models were significant. However, the lack of fit of both models were less than 0.05 indicated that the lack of fit was also significant. The reliability of the models was further investigated in the subsequent validity study. Besides, high value of correlation coefficient, R^2 (96.13% and 85.20%, respectively) and Adj-R^2 (92.64% and 71.88%, respectively) of both yield and DE models demonstrated that the models were well correlated to the experimental result obtained and suitable for predicting the relationship between independent variables and responses in the current study [7, 9]. From the R^2 value of both models, it indicated that only 3.87% of the variation in pectin yield response and 14.80% of the variation in DE response were not able to be explained by the models. In addition, *p*-value of each model term was used to determine the significance of the respective term where a *p*-value lower than 0.05 would indicate significant effect is imposed by the variable towards the response. In this study, it was found that the linear terms (*A* and *B*) and

Table 4 Central Composite Design (CCD) with experimental and predicted yield and DE of pectin extracted from orange peels

Run	Independent variables			Experimental responses		Predicted responses	
	A (°C)	*B*	*C* (min)	Yield (%)	DE (%)	Yield (%)	DE (%)
1	60	2	30	19.07	20.00	20.72	35.34
2	90	2	30	42.04	27.03	36.72	38.90
3	60	4	30	0.61	92.86	− 2.18	96.74
4	90	4	30	2.80	71.43	2.89	77.32
5	60	2	90	42.04	16.13	36.54	27.41
6	90	2	90	46.17	22.86	43.55	36.15
7	60	4	90	0.71	80.00	0.62	85.29
8	90	4	90	3.78	69.23	− 3.28	71.05
9	49.77	3	60	0.74	85.71	2.13	72.71
10	100.23	3	60	6.04	75.00	12.3	63.73
11	75	1.32	60	58.39	25.49	62.79	2.98
12	75	4.68	60	0.89	85.71	4.14	83.95
13	75	3	9.55	2.82	87.50	4.00	73.79
14	75	3	110.45	5.64	72.40	12.11	61.84
15	75	3	60	4.02	76.73	4.70	81.41
16	75	3	60	4.08	86.13	4.70	81.41
17	75	3	60	4.87	81.25	4.70	81.41
18	75	3	60	5.31	76.92	4.70	81.41
19	75	3	60	5.60	86.36	4.70	81.41
20	75	3	60	5.61	76.92	4.70	81.41

Table 5 The result of analysis of variance (ANoVA) for (a) pectin yield; and (b) DE

Source	Sum of squares	*df*	Mean square	*F*-value	*p*-value
(a) Pectin yield					
Model	6032.42	9	670.27	27.57	< 0.0001
A	124.74	1	124.74	5.13	0.0470
B	4151.95	1	4151.95	170.78	< 0.0001
C	79.37	1	79.37	3.26	0.1009
AB	59.62	1	59.62	2.45	0.1484
AC	40.32	1	40.32	1.66	0.2268
BC	84.63	1	84.63	3.48	0.0916
A^2	11.44	1	11.44	0.4706	0.5083
B^2	1491.06	1	1491.06	61.33	< 0.0001
C^2	20.34	1	20.34	0.8366	0.3819
Residual	243.11	10	24.31		
Lack of fit	240.50	5	48.10	92.20	< 0.0001
Pure error	2.61	5	0.5217		
Cor total	6275.53	19			
(b) Pectin yield					
R^2	0.9613				
Adj-R^2	0.9264				
(b) DE					
Model	11371.78	9	1263.53	6.40	0.0038
A	97.30	1	97.30	0.4925	0.4988
B	7915.05	1	7915.05	40.06	< 0.0001
C	172.20	1	172.20	0.8717	0.3725
AB	264.04	1	264.04	1.34	0.2745
AC	13.42	1	13.42	0.0679	0.7997
BC	6.16	1	6.16	0.0312	0.8634
A^2	313.55	1	313.55	1.59	0.2363
B^2	2594.12	1	2594.12	13.13	0.0047
C^2	333.09	1	333.09	1.69	0.2233
Residual	1975.57	10	197.56		
Lack of fit	1869.41	5	373.88	17.61	0.0034
Pure error	106.16	5	21.23		
Cor total	13347.35	19			
R^2	0.8520				
Adj-R^2	0.7188				

quadratic term (B^2) were significant in yield model whereas the significant terms for DE model were the linear term (B) and quadratic term (B^2).

Effect of extraction process variables on the response variables

The interactive effects of the independent variables on the yield and DE of pectin were studied by analyzing the 3D response surface graphs and contour plots in Figs. 2 and 3, respectively. In each illustration, the interactive effect between two independent variables and pectin response could be observed which the remaining independent variable was kept constant at the central level of the range studied (zero coded level).

Effect of extraction process variables on pectin yield

From Table 4, it was noted that different pectin yield was extracted at different extraction conditions, ranging from 0.61% (run no. 3) to 58.39% (run no. 11). Based on the ANoVA analysis tabulated in Table 5, the extraction process variables that showed significant influence on pectin yield was extraction temperature and solvent pH. The former (p-value of 0.0470) however was less significant compared to solvent pH (p-value was < 0.0001). Extraction time was regarded as not significant on pectin yield in this study. The significance level of these variables was in agreement with the previous studies on the pectin extracted from banana peels [40] and watermelon rind waste [37] where the pectin yield was mainly affected by temperature and pH but less affected by extraction time.

As shown in Fig. 2a, higher pectin yield was extracted from the orange peels when extraction temperature increased from 60 to 90 °C, concurrently with decreased pH for a fixed extraction period. The same effect of temperature on the yield was reported in the previous optimization studies on pectin extracted from carrot pomace [23] and melon peel [45]. However, there was only a slight increase in yield when temperature was increased and as the extraction time was prolonged at constant pH 3 (Fig. 2b). At higher temperature, the increase in pectin yield could be attributed to a higher diffusivity of solvent into the cell tissues (fruit peels), thereby increasing the mass transfer of pectin into the solvent [19, 49]. Moreover, Dao et al. [9], and Cho and Hwang [6] explained that higher temperature could facilitate the conversion of insoluble protopectin into soluble pectin, thus increasing the amount of pectin extracted.

In addition, solvent pH was an identified as important factor that caused significant effects towards both pectin yield and DE in this study. This was consistent with numerous studies such as pectin extraction from sweet potato peels [19], muskmelon [56] and citron peel [41]. Figure 2a and c showed a similar trend in the response surface graphs in which a significant increase of pectin yield was observed with decreasing pH from 4 to 2. A positive effect on pectin yield was also shown in the previous studies by reducing solvent pH [23, 40, 41]. One of the plausible reasons could be lower pH promoted the hydrolysis reaction of insoluble protopectin, leading to a higher amount of soluble pectin [10]. The hydrolysis reaction of protopectin was facilitated at a decreasing pH due to the presence of higher concentration of hydrogen ion in the solvent. At a higher concentration of hydrogen ion, the repulsion of polysaccharide molecules was inhibited owing to the hydrolysis reaction that resulted loss of charges in the carboxylate groups by converting the

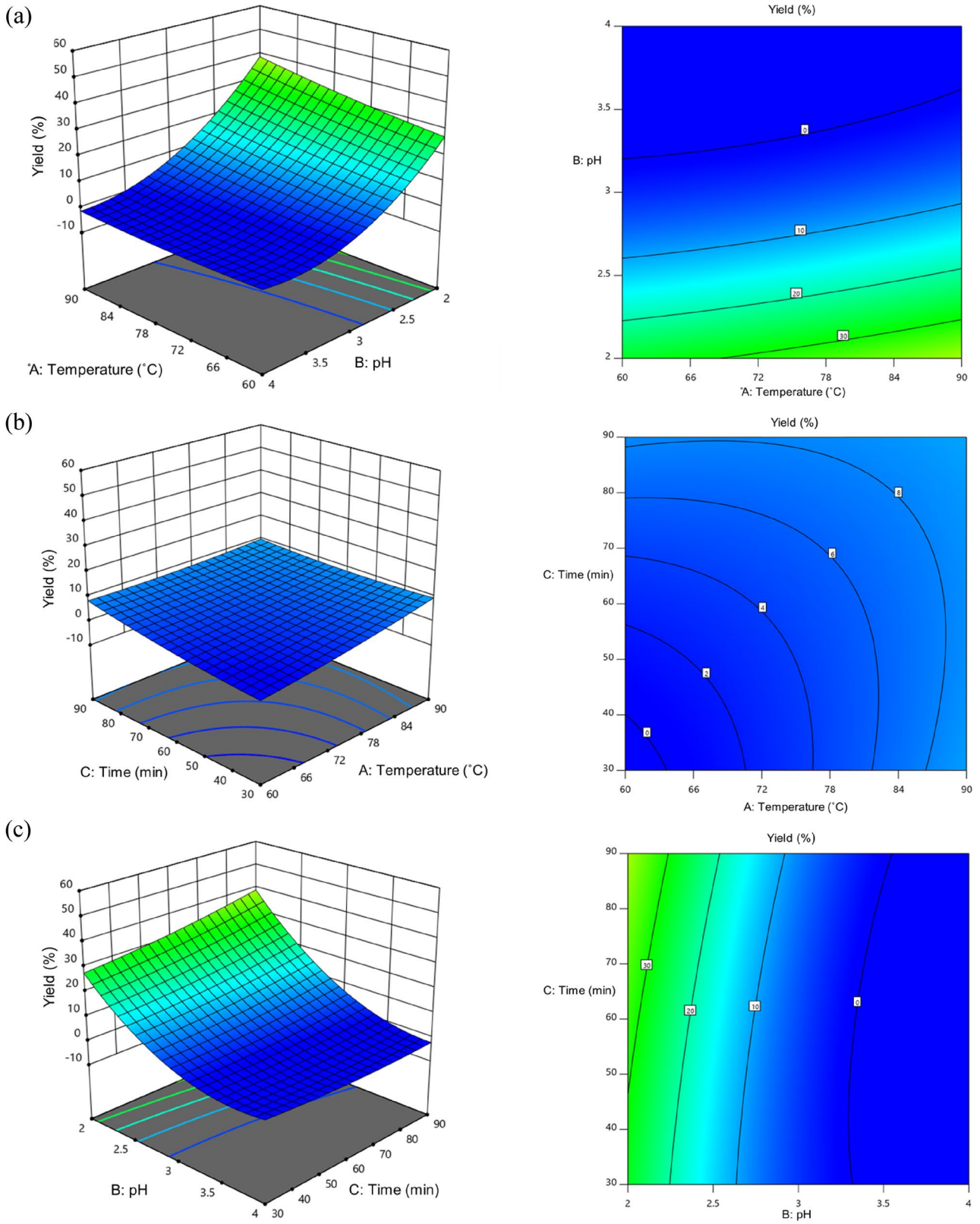

Fig. 2 3D response surface plot and contour plot of the interactive effect of two independent variables on pectin yield: **a** Extraction temperature and solvent pH at 60 min. **b** Extraction temperature and extraction time at pH 3. **c** pH and extraction time at 75 °C

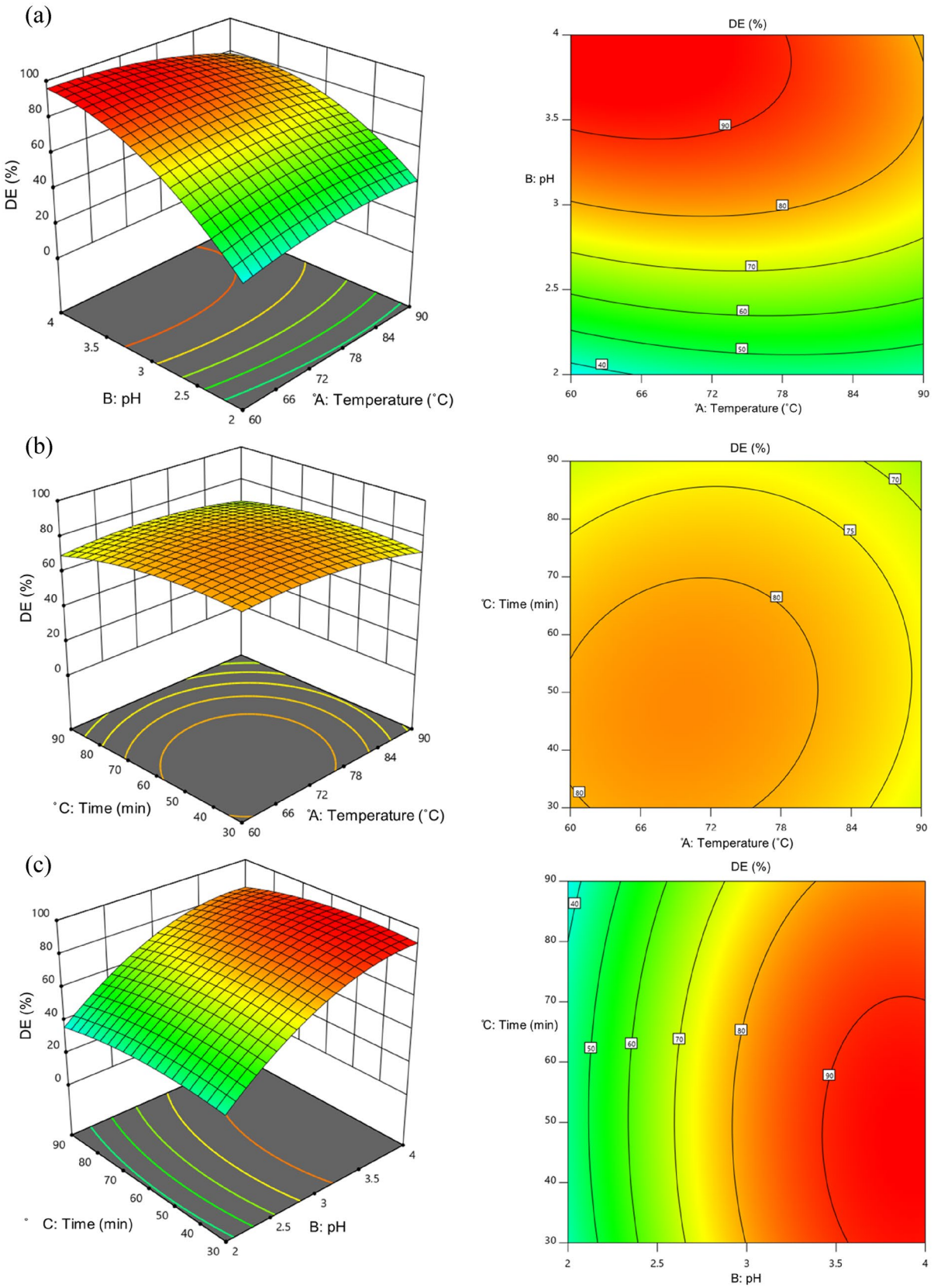

Fig. 3 3D response surface plot and contour plot of the interactive effect of two independent variables on pectin DE. **a** Extraction temperature and solvent pH at 60 min. **b** Extraction temperature and extraction time at pH 3. **c** Solvent pH and extraction time at 75 °C

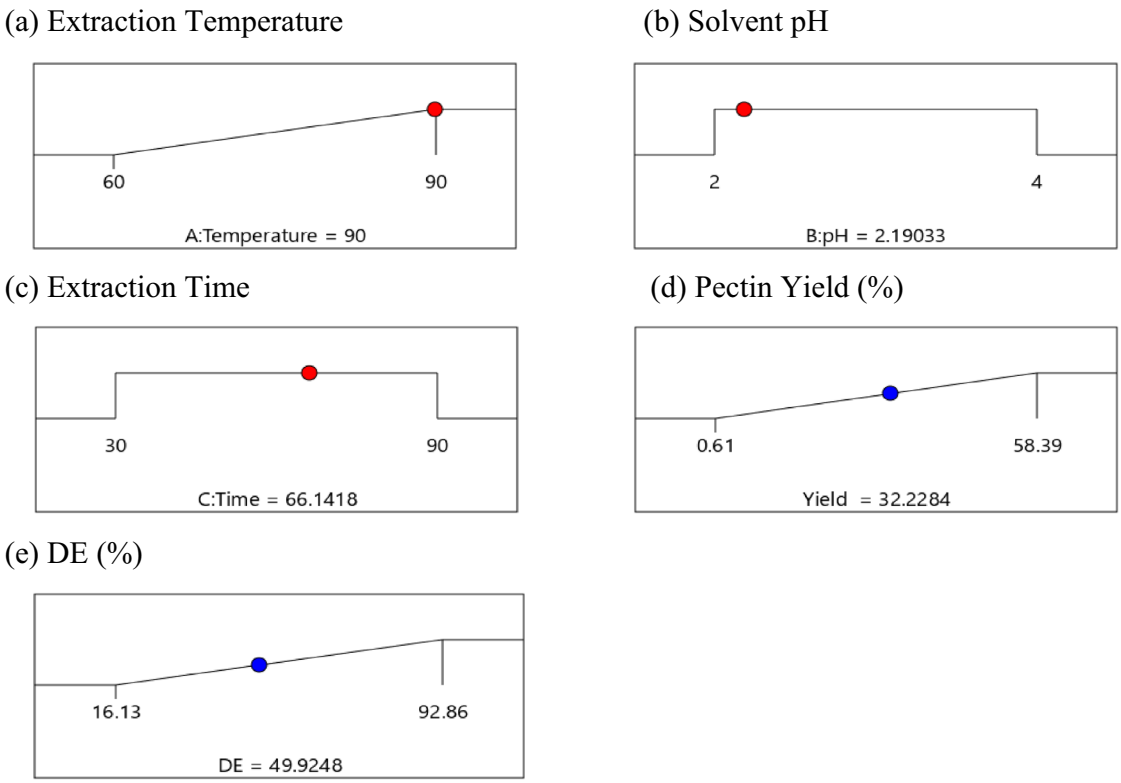

Fig. 4 Ramp plot of optimal extraction condition for **a** Extraction temperature. **b** Solvent pH, **c** Extraction time; and the predicted optimal responses. **d** Pectin yield and **e** DE

hydrated carboxylate groups into hydrated carboxylic acid groups [10, 13]. Consequently, the decreasing repulsion between polysaccharide molecules led to a better gelation behavior of pectin and thus improving the efficiency of pectin precipitation.

As for the effect of extraction time, a greater effect was shown in the interaction between the extraction time and solvent pH as shown in Fig. 2c compared to the interaction between extraction time and temperature as shown in Fig. 2b. Although the effect was not obvious, it could be observed that higher pectin yield was obtained when the extraction process was carried out at a longer duration. A recent study by Méndez et al. [37] also demonstrated that extraction time was not significant to enhance pectin yield extracted from watermelon rind waste. Nonetheless, there were some previous work reported that longer extraction time could significantly increase the yield of pectin such as pectin extraction from cocoa husks [5] and carrot pomace [23]. The authors correlated the results to the longer period provided to allow the transfer of pectin from cell tissues into the solvent, hence higher yield was extracted.

Effect of extraction process variables on DE

From the optimization results, the DE of extracted pectin was in the range of 16.13% (run no. 5) to 92.86% (run no. 3), indicated that both HM and LM pectin were extracted from orange peels at different extraction conditions. Figure 3 shows the interaction effect of the process variables on pectin DE whereby the greatest effect on DE was exhibited by solvent pH. Although the effect on DE caused by extraction temperature and time was not significant, it could be observed that higher pectin DE was obtained when the solvent pH was higher, but shorter extraction time and lower extraction temperature. Similar relationship between the process variables and pectin DE was reported in the literature particularly on pectin extraction from citron peel [41], mango peel [50] and watermelon rind waste [37]. The authors reported that DE of pectin increased with increasing solvent pH, but reduction of extraction time and temperature which was similar to the trend observed in this study. As pectin was extracted at lower pH and higher temperature for a longer duration, the harsh condition could accelerate de-esterification of polygalacturonic chains, thereby decreased the pectin DE [41].

In the present study, solvent pH was the most significant variable identified from the ANoVA result which influenced

pectin DE the most. This was in agreement with the work of Dranca and Oroian [11], and Jafari et al. [23] where the authors reported that pH caused a greater influence on the pectin DE as compared to other process variables in their study. From Fig. 3a and c, a significant increase in DE value was observed with increasing solvent pH from 2 to 4. The positive relationship coincides with results obtained by Pasandide et al. [41], Dranca and Oroian [11], and Sangheetha et al. [50]. At higher pH, availability of hydrogen ion for de-esterification of pectin would be limited, hence the DE value would be higher [50]. Based on the relationship between extraction temperature and solvent pH depicted in Fig. 3a, higher pectin DE was obtained with increasing solvent pH concurrently with decreasing extraction temperature. This could be due to the lack of thermal energy to hydrolyze the ester bonds in pectin structure at lower temperature, thus preventing de-esterification of pectin from happening [50]. Following that, the interacting effect between solvent pH and extraction time on pectin DE in Fig. 3c also showed that higher solvent pH with shorter extraction time led to a higher pectin DE. As the extraction duration was reduced, shorter time was allowed for the occurrence of de-esterification of pectin, thus reducing the frequency of de-esterification reaction to occur [50]. In addition, it could be seen in Fig. 3b that insignificant increase of pectin DE was observed as the extraction temperature was lowered and the extraction time was shortened. On the contrary, a lower pectin DE was obtained with the decreasing solvent pH, increasing extraction temperature and longer extraction time. This phenomenon could be due to the harsh extraction conditions which stimulated the de-esterification of polygalacturonic chains [23, 45, 60].

Validation of optimum extraction conditions

The optimum conditions were predicted by the Stat-Ease Design Expert version 11.0 software to be: extraction temperature at 90˚C, acidified solvent with pH 2.19 and extraction time of 66 min, as illustrated by the ramp plot in Fig. 4. At this optimized extraction conditions, the predicted yield and DE of extracted pectin was 32.23% and 49.92%, respectively. Pectin extraction was performed in triplicate at the suggested optimum conditions to validate the reliability of the models developed. The results obtained are tabulated in Table 6.

The experimental result of pectin yield obtained at the predicted optimum conditions was 35.20 ± 0.39% whereas the DE was 45.43 ± 0.39%. The results were close to the model prediction, with percentage error less than 10%, thus indicating that the developed models were feasible to predict the yield and DE of pectin extracted from orange peels [20]. It is worth mentioning that the optimum orange peel pectin yield obtained in this study was similar to some previous studies such as Zanella and Taranto [63] who reported that 38.21% of pectin yield was extracted from the albedo (white and spongy substance of orange peels) and 28.07 ± 0.67% of pectin yield was extracted from sour orange peels by Hosseini et al. [21]. Apart from that, the pectin yield obtained from orange peels by using ultrasound assisted extraction (UAE) was 26.87% [22] and microwave assisted extraction (MAE) was 19.24% [35]. Hence, the pectin yield obtained in this study was comparable to or even higher than the reported value in the literature.

On the other hand, it was important to note that the DE of the extracted pectin at optimal conditions was below 50%, thereby indicating that the proposed optimal conditions was more suitable for the extraction of LM pectin. In general, HM pectin could also be obtained from the extraction of orange peels as proven in the comparison of extracting solvent and experimental results at several extraction conditions (Table 4). Based on previous works that were also done on the extraction of orange peels pectin, DE of the pectin extracted was above 50% which indicated the orange peels pectin extracted was HM pectin [13, 53, 57]. This difference in DE value or type of pectin extracted from orange peels could be due to the different extraction conditions that have been employed compared to the present study. Similar scenario was encountered by Sangheetha et al. [50] who reported that both HM and LM pectin were extracted from mango peels by varying the variables of solvent pH, extraction temperature and time.

Table 6 Experimental result of pectin yield and DE obtained under optimum conditions

Experimental trial	Pectin yield (%)	DE (%)
1	35.53	44.94
2	34.65	45.45
3	35.42	45.90
Average	35.20	45.43
Predicted value	32.23	49.92
Error (%)	9.22	8.99

Conclusion

In this study, the effect of acid solvent (acetic acid, hydrochloric acid, citric acid and nitric acid) on the yield and DE of pectin extracted from orange peels was investigated. Citric acid was chosen as the best extracting solvent to use in the optimization study because it produced the highest pectin yield and optimum pectin DE (at the range of HM) when compared to other acids. For the multi-objective optimization of pectin extraction from orange peels using

RSM, the developed models were validated by experimental results with less than 10% error. It was discovered that the extraction process variables had an opposite effect on pectin yield and DE. As a result, the optimized conditions were better suited for extracting LM pectin (DE < 50%) from orange peels. The findings of this study confirmed that extraction conditions could affect the DE value of pectin, which was one of the important properties influencing its gelling abilities.

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Data availability (data transparency) All data and materials provided in the manuscript originated from the experimental work performed by the authors

Code availability (software application or custom code) Not applicable.

Declarations

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