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OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF PECTIN FROM PASSION FRUIT (*Passiflora edulis* Sims) PEEL WITH ALKALINE SOLUTION USING RESPONSE SURFACE METHODOLOGY

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Abstract

Pectin production from passion fruit peel was carried out using microwave-assisted extraction. The extraction factors (NaOH concentration, liquid to solid (LS) ratio, and extraction time) were optimized using response surface methodology (RSM), which was applied to optimize the yield, purity, and degree of esterification (DE) of pectin by implementing central composite face (CCF) design. The response surface showed the relationships between the independent factors, and the DE value was not affected by these factors. The optimal extraction process was obtained at NaOH concentration of 183 mM, LS ratio of 41 mL/g, and time of 7.5 min. Under these conditions, the model predictions for the yield, purity, and DE value of pectin from passion fruit peel were 14.31%, 87.25%, and 92.94%, respectively, which were verified experimentally at 14.29%, 87.1%, and 93.7%, respectively. This study demonstrated the great efficiency of applying experimental design to characterize the operational parameters influencing the extraction process.

INTRODUCTION

Passion fruit (*Passiflora edulis*) belongs to the genus *Passiflora*. This plant originates from Brazil, Paraguay, Argentina and is considered of great economic value. The species *Passiflora edulis* Sims is currently cultivated widely in many tropical and subtropical countries, especially Vietnam. All parts of this plant were functional; for instance, the leaf extract treats some symptoms of alcoholism, anxiety, migraine, nervousness, and insomnia; a drink from the flower treats asthma and bronchitis, while seed oil is used as a lubricant and massage oil [1]. In addition, the pulp can be used for juicing or jam processing with unique sweet and sour flavors because it is rich in vitamin C [2], total carotenoids [3], and also has antioxidant properties [4]. Besides, some previous studies have pointed out that peel of the passion fruit has a high pectin content; for instance, according to Liew et al. [5] and Oliveira et al. [6], the pectin yields are 7.12% and 12.67%, respectively. These authors extracted pectin from the passion fruit by various methods such as enzyme-assisted extraction (EAE) or ultrasound-assisted extraction (UAE). This is also a potential material in the future for many foods, such as edible film [7], fruit-filling [8], or food packaging [9]. However, in Vietnam, during the passion fruit season, the peel is considered a solid waste, leading to serious environmental problems. Hence, the conversion of peel into valuable compounds decreases waste and supplies an economically feasible alternative material for the food industry.

Nowadays, there are many methods by which pectin is extracted from plants in high yield and with great properties; for instance, conventional methods, EAE [5], UAE [6], etc. However, the problems of these methods are the high energy and solvent consumption and long extraction time. An alternative method is needed to enhance pectin extraction yield. Microwave-assisted extraction (MAE) seems to be a reliable solution compared to other techniques. In fact, recent studies reported using MAE to extract pectin dragon fruit peel [10], phenolic compounds from *Polygonum multiflorum* Thunb. roots [11], total triterpenoid saponins from *Ganoderma atrum* [12], etc.

Hence, MAE was the choice for this study to isolate pectin from passion fruit peel.

The aim of the present study was to clarify the relationships between the independent variables (NaOH concentration, LS ratio, and extraction time) and responses (yield, purity, and degree of esterification (DE) of pectin) and optimize the conditions for pectin extraction from passion fruit peel. Therefore, response surface methodology (RSM) was carried out using a central composite face (CCF) design.

MATERIALS AND METHODS

Materials

The fruits of *P. edulis* used in this study were harvested from Daklak province (Vietnam). All other chemicals used were of analytical reagent grade.

Sample Preparation

Briefly, the passion fruit peel was washed with tap water, the purple skin removed and the peels separated from the fruit. After that, the peel was dried in a hot-air dryer (80ºC) to a moisture content of approximately 5% and ground for 3 min (Panasonic MX-V310KRA, China) to yield a fine powder (0.8 mm particle size). Finally, the samples were vacuum-packed in a polyethylene (PE) bag and stored at room temperature (29±2ºC).

Pectin Extraction Process

Pectin from passion fruit was extracted using a microwave apparatus (Whirlpool model MWX201BL, China) with a microwave power of 376 W. A mass of 5 g dry sample was soaked in NaOH solution under microwave heating, and all experiments were performed at set solvent concentrations, LS ratios, and irradiation times according to the experimental design given in Tables 1 and 2. The extract was then cooled to room temperature (29±2ºC) and filtered through cloth to remove the residue. Alcohol (99%, v/v) was added to the filtrate (filtrate to alcohol ratio of 1:1, v/v) and the mixture was left for 1 h to precipitate the pectin completely. The crude pectin was filtered and purified with alcohol (70%, v/v) solution. Finally, it was dried at 80ºC in a hot-air oven to constant weight. The dried crude pectin was packed and stored for subsequent analysis.

Determination of Crude Pectin Content

The percentage crude pectin yield (Y) was calculated from the following equation:

$$Y = \frac{m_1}{m_0} \times 100\% \quad (1)$$

m_1: Mass of crude pectin (g)
m_0: Mass of dried sample (g)

Determination of Purity of Pectin

According to the procedure of Quoc [13], 0.15 g of crude pectin was soaked in 100 mL of 0.1 N NaOH for 7 h. Then, 50 mL of 1 N CH_3COOH was added to the mixture. After 5 min, 50 mL of 2 N $CaCl_2$ was added to the mixture and left to stand for 1 h, after which the solution was boiled for 5 min, filtered by Whatman filter paper (No. 4) under vacuum, and the received residue dried to constant weight. The calcium pectate obtained was washed with hot water until the solution's Cl– ions were not detected and dried for 2 h at 105°C. The purity of pectin was determined according to the equation:

$$P = \frac{m_{calcium\ pectate} \times 0.92}{m_{crude\ pectin}} \times 100\% \quad (2)$$

P: Purity of pectin (%)
$m_{calcium\ pectate}$: Mass of calcium pectate (g)
$m_{crude\ pectin}$: Mass of crude pectin (g)
0.92: Coefficient of pectin in calcium pectate of 92% by volume

Determination of Degree of Esterification of Pectin

The DE of the pectin was determined by the method of Pinheiro et al. [14] with some slight modifications. Briefly, the dried pectin (0.15 g) was moistened with ethanol, dissolved in 20 mL of distilled water at 40ºC, and stirred slightly for 2 h. Then, the mixture was titrated with 0.1 N NaOH in the presence of phenolphthalein to determine the number of free carboxyl groups. The result was recorded as the initial titer (V_1). After that, 10 mL of 0.1 N NaOH solution was added to a neutralized sample of polygalacturonic acid. The mixture was stirred for 2 h to saponify the esterified carboxyl groups of the polymer. Then, 10 mL of 0.1 N HCl was added to neutralize the NaOH and excess HCl in the sample was further titrated with 0.1 N NaOH. This titration volume was recorded as the saponification titer (V_2). The DE value was calculated from the following formula:

$$DE = \frac{V_2}{V_1 + V_2} \times 100\% \quad (3)$$

Experimental Design

In the present study, the experimental design was conducted using RSM. Second-order CCF design with three factors and three levels was chosen to investigate and optimize the effect of independent variables (NaOH

concentration, x_1; LS ratio, x_2; and extraction time, x_3) on the dependent variables, including yield (Y_1), purity (Y_2), and DE value (Y_3) of pectin. Based on our preliminary experiments, the experimental design is shown in Table 1. Experimental data were fitted to a second-order polynomial equation; the regression model of this equation was as follows:

$$Y_r = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} . \sum_{j=2}^{n} \beta_{ij} x_i x_j \quad (4)$$

where β_0, β_i, β_{ii}, and β_{ij} are regression coefficients; Y_r are the responses; x_i and x_j are the independent variables.

Table 1. Experimental and coded levels of three variables used for pectin extraction in the CCF design

Factors (Independent variables)	Code Symbols	Levels		
		-1	0	1
NaOH concentration (mM)	x_1	150	180	210
LS ratio (mL/g)	x_2	30:1	35:1	40:1
Extraction time (min)	x_3	5	7	9

Statistical Analysis

Statistical analysis was performed using Excel (version 14.0, Microsoft Corp, USA). Significant differences between the means were specified at ($p_{value} \leq 0.05$). Experimental design, data analysis and three-dimensional (3D) response surface plots were designed, analyzed, and plotted using Modde 5 software (version 5.0, 1999, Umetrics AB, Umea, Sweden).

RESULTS AND DISCUSSION

Analysis of the Model

The extraction of pectin from passion fruit peel was optimized through the RSM approach. A fixed microwave power (376 W) was chosen. The results of pectin yield, purity, and DE value for all runs are displayed in Table 2. The independent variables and the responses were fitted to the second-order polynomial equation and the goodness of fit was determined.

Table 2. CCF design with the experimental values and predicted values

Run	Independent variables			Experimental results			Predicted results		
	x_1	x_2	x_3	Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_1 (%)	Y_2 (%)	Y_3 (%)
1	150	30:1	5	11.7	85.9	93.7	11.72	85.74	93.45
2	210	30:1	5	12.9	84.5	93.7	12.90	84.68	93.66
3	150	40:1	5	12.1	85.8	92.6	12.03	85.94	92.64
4	210	40:1	5	13.2	84.6	92.6	13.26	84.53	92.82
5	150	30:1	9	12.8	85.5	92.7	12.70	85.59	92.50
6	210	30:1	9	13.8	84.8	92.7	13.83	84.68	92.68
7	150	40:1	9	13.1	85.5	93.7	13.06	85.34	93.68
8	210	40:1	9	14.3	83.9	93.6	14.24	84.08	93.83
9	150	35:1	7	13.1	86.7	92.7	13.29	86.78	93.15
10	210	35:1	7	14.5	85.8	93.7	14.47	85.62	93.33
11	180	30:1	7	13.6	86.9	92.6	13.65	86.90	93.04
12	180	40:1	7	13.9	86.8	93.6	14.01	86.70	93.21
13	180	35:1	5	13.1	87.1	92.6	13.09	87.00	92.61
14	180	35:1	9	13.9	86.7	92.6	14.07	86.70	92.65
15	180	35:1	7	14.3	87.5	93.6	14.16	87.39	92.92
16	180	35:1	7	14.3	87.6	93.5	14.16	87.39	92.92
17	180	35:1	7	14.2	86.9	91.8	14.16	87.39	92.92

x_1, x_2, and x_3 are NaOH concentration (mM), LS ratio (mL/g), and extraction time (min), repectively; Y_1, Y_2, and Y_3 are the yield (%), purity (%), and DE value (%) of pectin, repectively.

Table 3 shows the analyses of variance performed to evaluate the goodness of fit and the significance of the linear, quadratic, and interaction influences of the independent variables on the responses. In this study, among the regression models, only the p_{value} of Y_1 and Y_2 were <0.05, which means that there was a statistically significant multiple regression relationship between the independent variables and the response variable. In addition, the R^2 and R^2_{adj} values of Y_1 and Y_2 were >0.9, indicating the suitability of the applied regression model [15]. The response surface models could explain more than 98.6% and 97.4% of the variation of the studied response variables, respectively. Besides, there was no significant lack of fit in all the response variables (lack of fit > 0.05), indicating that the model was sufficiently accurate to predict the response variations.

Table 3. Analysis of variance (ANOVA) for yield, purity and DE value of of the pectin extracted from the passion fruit peel

Factors	Y_1		Y_2		Y_3	
	Coefficient	p_{value}	Coefficient	p_{value}	Coefficient	p_{value}
Constant	14.161	7.883×10^{-15}	87.394	1.957×10^{-18}	92.923	5.591×10^{-16}
x_1	0.590	3.834×10^{-6}	-0.580	0.000	0.090	0.674
x_2	0.180	0.006	-0.10	0.283	0.070	0.743
x_3	0.490	1.331×10^{-5}	-0.150	0.125	0.010	0.962
x_1^2	-0.281	0.015	-1.190	0.000	0.311	0.459
x_2^2	-0.331	0.007	-0.590	0.009	0.211	0.612
x_3^2	-0.581	0.000	-0.540	0.014	-0.289	0.489
x_1x_2	0.013	0.813	-0.088	0.393	-0.012	0.958
x_1x_3	-0.012	0.813	0.038	0.708	-0.012	0.958
x_2x_3	0.012	0.813	-0.113	0.280	0.512	0.061
Q^2	0.905		0.771		-0.802	
R^2	0.986		0.974		0.487	
R_{adj}^2	0.968		0.941		-0.172	
p_{value}	0.000		0.000		0.675	
Lack of fit (F)	0.111		0.867		0.949	

x_1, x_2, and x_3 are NaOH concentration (mM), LS ratio (mL/g), and extraction time (min), repectively; Y_1, Y_2, and Y_3 are the yield (%), purity (%), and DE value (%) of pectin, repectively.

However, for the DE values (Y_3), the R^2 and R^2_{adj} values were 0.487 and -0.172, respectively. This shows that good fit was not obtained and most of the variability in responses was not explained by the model. In other words, the DE values did not depend on the changes in extraction factors and the DE values only fluctuated from 91.8% to 93.7%. Thus, it is unnecessary to determine the regression equation for the DE values.

A much better indication of the usefulness of a regression model is given by the Q^2 parameter, called the goodness of prediction that estimates the predictive power of the model. In this case, only the Q^2 parameters for Y_1 and Y_2 were >0.5 (0.905 for Y_1 and 0.771 for Y_2); besides, R^2 and Q^2 were not separated by more than 0.3. This proved that the models (Y_1 and Y_2) have good predictive power [16].

$$Y_1 = 14.161 + 0.59x_1 + 0.18x_2 + 0.49x_3 - 0.281x_1^2 - 0.331x_2^2 - 0.581x_3^2 + 0.013x_1x_2 - 0.012x_1x_3 + 0.012x_2x_3$$

$$Y_2 = 87.394 - 0.580x_1 - 0.1x_2 - 0.015x_3 - 1.19x_1^2 - 0.59x_2^2 - 0.54x_3^2 - 0.088x_1x_2 + 0.038x_1x_3 - 0.113x_2x_3$$

For the pectin extraction yield, at the linear terms, NaOH concentration (x_1), LS ratio (x_2), and extraction time (x_3) had positive influences on the pectin yield. NaOH concentration had the most significant influence on pectin yield followed by extraction time and LS ratio, respectively. At the quadratic terms, all independent variables had a negative influence on the response (the strongest effect was by extraction time). In particular, there were no interactions between all variables (p_{value}>0.05) (Table 3).

For the pectin purity, the p_{values} of x_1, x_1^2, x_2^2, and x_3^2, were all <0.05, indicating that their coefficients were significant. They had a negative influence on pectin purity. NaOH concentration (x_1) played the most important role in both linear and quadratic terms. All other variables (x_2, x_3, x_1x_2, x_1x_3, and x_2x_3) of the model were not significant (p_{value}>0.05; Table 3), indicating that they did not affect the purity of the pectin obtained.

Response Surface Plots

The 3D-response surfaces and contour plots of pectin yield (Y_1) and purity (Y_2) are shown in Figures 1 and 2. As shown in Figures 1 and 2, the yield and purity of pectin were changed following the change of NaOH concentration (%), LS ratio (mL/g), and extraction time (min) to a certain value. The yield of pectin extraction significantly increased

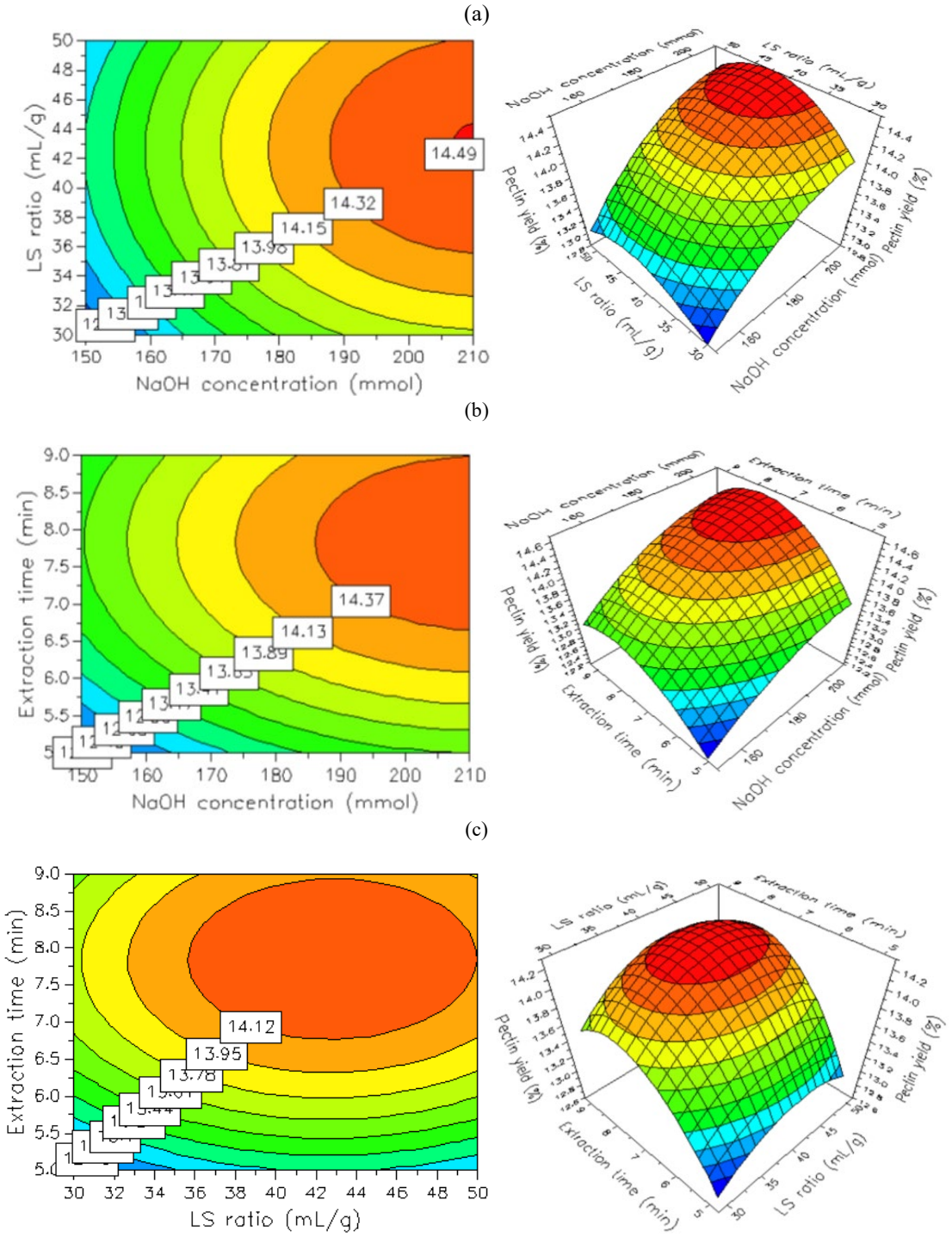

Figure 1. Contour and response surface plots showing the effects of investigated factors on pectin yield and their interactions: a) Time constant at 7 min, b) LS ratio constant at 35 mL/g, c) NaOH concentration constant at 180 mM.

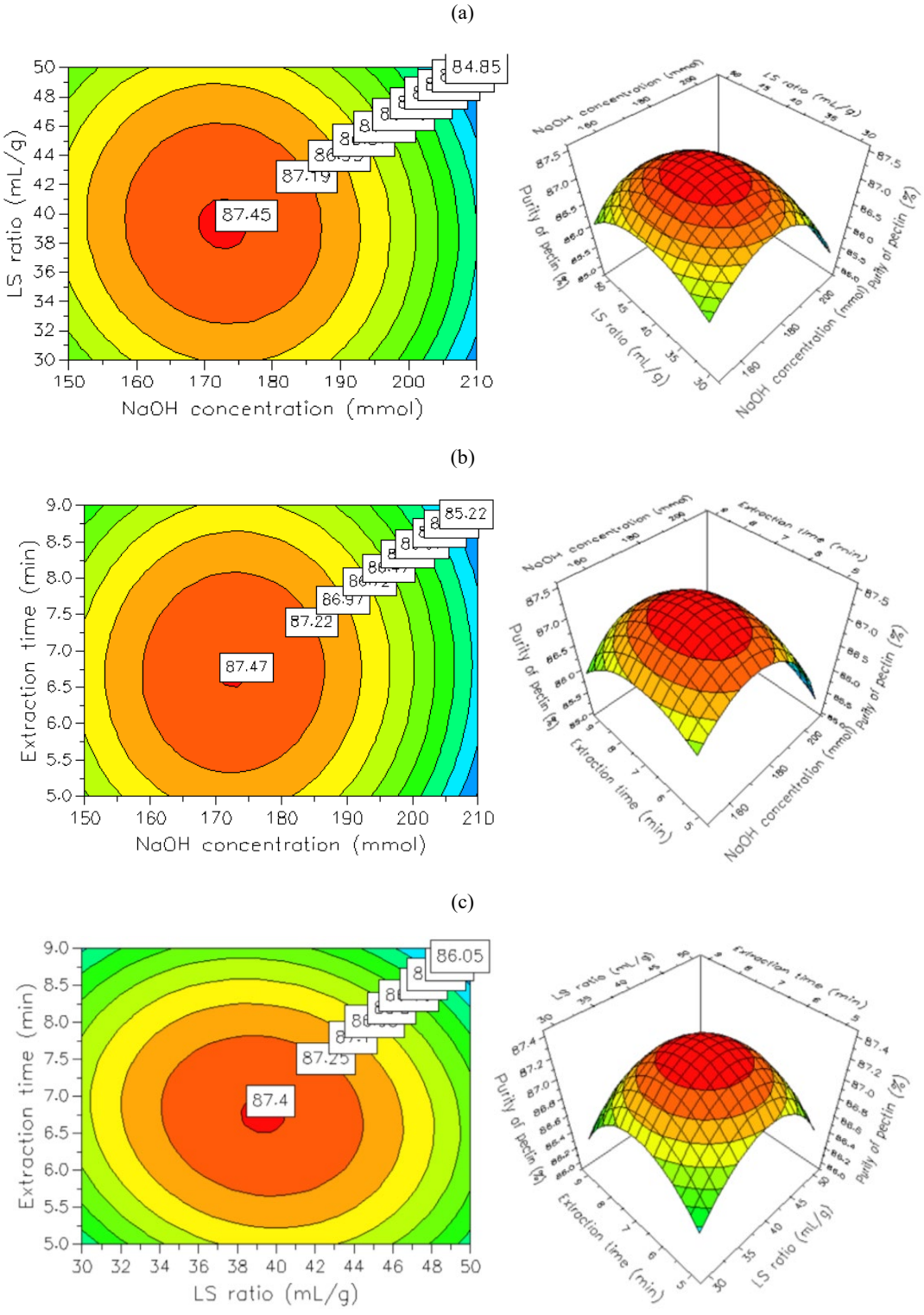

Figure 2. Contour and response surface plots showing the effects of investigated factors on the purity of pectin and their interactions: a) Time constant at 7 min, b) LS ratio constant at 35 mL/g, c) NaOH concentration constant at 180 mM.

with an increase in heating time, LS ratio, and NaOH concentration. If the values of these variables increased beyond an optimal point, the yield and purity of pectin decreased in all cases. Kulkarni and Vijayanand [17] have already reported similar results when isolating pectin from passion fruit peel. In our study, the yield peaked at 14.49% at an extraction time of 7 min. NaOH concentration ranged from 205 to 210 mM and LS ratio from 42 to 44 mL/g (Figure 1a).

Figure 2 points out that the contour plots seemed to be elliptical or circular. The pectin purity increased with NaOH concentration, extraction time, and LS ratio. However, this increase had a limited extent; further increases to higher values of the parameters resulted in decreased pectin purity. This observation is similar to Quoc [13], who extracted pectin from pomelo peel by microwave assistance. The maximum purity reached in this study was 87.47% at LS ratio of 35 mL/g, NaOH concentration between 170 and 175 mM, and extraction time from 6.5 to 6.7 min (Figure 2b).

In general, all independent variables affected the responses strongly. Pectin yield was reduced after a maximum point, possibly explained by the influence of solvent, which may have destroyed the glycoside bonds and ester bonds of pectin, which led to a lower yield. For the LS ratio, the optimum ratio may improve the pectin extraction. If the LS ratio is too low, the pectin in the sample was not extracted completely. On the contrary, a high LS ratio will lead to high process cost and longer extraction times. In addition, an increase in extraction time could lead to an increase in the destruction of pectin, causing a reduction in molecular weight and the gelling properties of the pectin [18].

Prediction and Experimental Validation

As seen in Table 4, the model from the CCF design revealed that the optimum conditions for maximizing both yield and purity of pectin were NaOH concentration of 183.37 mM, an LS ratio of 40.71 mL/g, and an extraction time of 7.43 min. However, considering the operational convenience of the microwave system, optimal values of variables were determined at NaOH concentration of 183 mM and an LS ratio of 41 mL/g for 7.5 min. Under these extraction conditions, the yield, purity, and DE value accounted for 14.29%, 87.1%, and 93.7%, respectively. The differences between experimental data and theoretical results are insignificant. This demonstrated that the proposed models were considered to be accurate and reliable for predicting the yield and purity of pectin of passion fruit by MAE. In general, pectin yield in this study was higher than that from other materials, for instance, orange peel (13.32%) [19], mangosteen rind (1.16%) [20], Chinese quince fruit (10.49%) [21], etc. Besides, the DE value obtained was 93.7%; thus this is high-methoxyl pectin (HMP, DE>50%) [10]. Compared to the same material, Seixas et al. [22] isolated pectin from passion fruit peel using MAE with nitric and acetic acids as solvents, both the extraction yield and DE values were lower than those of this study (13%, 64.15% for nitric acid and 12.9%, 64.56% for acetic acid, respectively). For using the UAE technique, results recorded by Oliveira et al. [6] were also lower than our results (the yield and DE value were 12.67% and 60.36%, respectively). These differences were due to various sources of material, extraction methods, especially the type of solvent.

Table 4. Optimum conditions, predicted and experimental values of responses on pectin extraction

Independent variables			Responses	Predicted values	Experimental values
x_1	x_2	x_3			
183.37	40.71	7.43	Y_1	14.31	14.29±0.02
			Y_2	87.25	87.1±0.44
			Y_3	92.94	93.7±0.22

x_1, x_2, and x_3 are NaOH concentration (mM), LS ratio (mL/g), and extraction time (min), respectively; Y_1, Y_2, and Y_3 are the yield (%), purity (%), and DE value (%) of pectin, respectively.

These findings showed that we could completely isolate pectin of high yield, purity, and DE value from passion fruit peel using MAE and alkaline solution. This material seems to be a cheap and abundant source of pectin for the food industry in the future.

CONCLUSION

The evaluation of extraction conditions for pectin production was made using a CCF design. The effects of three independent factors were studied and all were found to be significant variables for pectin production from passion fruit peel. The DE values seemed to be unchanged during the extraction process. For maximum extraction efficiency, the optimum extraction parameters were fixed at a NaOH concentration of 183 mM, an LS ratio of 41 mL/g, and an extraction time of 7.5 min, respectively.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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