

## Research Article

# Comparative Studies of Combined Influence of Variables on the Esterification Degree of Pectin Extracted by Sulfuric Acid and Citric Acid

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The influence of extraction variables on degree of esterification (DE) of pectin was studied due to the significant effects of DE on the properties of the pectin-based functional biomaterials. The extracting agents (sulfuric acid and citric acid), pH value of extraction solution, extraction time, and temperature were selected to study the hydrolysis reaction of carboxylic acid ester by response surface methodology (RSM). The hydrolysis reaction occurred more violently in the weak organic acid solution than that in the strong inorganic acid solution. The DE was significantly influenced by pH of extraction solution, extraction time, and temperature through the analysis of variance. In addition, the values of DE reduced with the increasing of extraction temperature, lapse of time, and decreasing of pH value. What is more, the effects of extraction conditions on the yield, monosaccharide composition, content of protein, and ferulic acid were also studied. It is evident that the lower pH value contributed to higher extraction yield. The content of Total CH and GalA of pectin extracted by sulfuric acid were higher than that of pectin extracted by citric acid, and the content of monosaccharide showed an opposite trend.

## 1. Introduction

Pectin is a complex of polysaccharide which exists in the cell wall of almost all higher plants [1]. Due to gelling, emulsifying, thickening, and stabilizing properties, pectin has been widely used in the food industry to improve food products in texture, taste, and appearance [2]. The commercial pectin is usually extracted from the by-product of citrus and apple. Sugar beet pulp (SBP) is the by-product from sugar industry and has been proved to be the potential raw materials for pectin extraction. Nowadays many advanced methods, including ultrasonic-assisted extraction process [3], microwave-assisted extraction process [4], and electromagnetic-induced heating process, [5] have been developed to extract pectin from numerous raw materials [6, 7]. However, the traditional heating process is still the common technology in the current pectin industry [8]. By now, plenty of published data indicate that the extraction conditions significantly influence the yield, degree of esterification (DE), and content of the monosaccharides and protein. The chemical structure and the physico-chemical properties of pectin dominate its corresponding functional

properties, including the gelling, emulsifying, thickening and stabilizing properties, and biological activity [2].

The DE is one of the most important indices of pectin, which directly determined the functional properties of the pectin. Generally, pectin can be divided into two groups based on the value of DE. The high-ester and low-ester pectins are denoted by the DE values higher or lower than 50% [2]. The low-ester pectin can form a gel in the presence of divalent metal ions within a wide range of pH values. The gel process could be described by the “egg-box” model (shown in Figure 1) which was first suggested for alginates [9]. The number of free carboxyl obviously affects liganacy and influences the steady of sexadentate chelate as a result [10]. In addition, the content of the free carboxyl group can easily change the hydrophilia of the pectin account for the hydrolyzing of  $H^+$ . Consequently, the DE of pectin significantly influence the gelling, emulsifying, thickening, and stabilizing properties [11, 12].

The essence of the DE is the percentages of ester carbonyl group. In the extraction process, the hydrolysis reaction of methyl ester occurred when the acids are employed as the catalyst. The types of acid, pH value of the extraction solution,

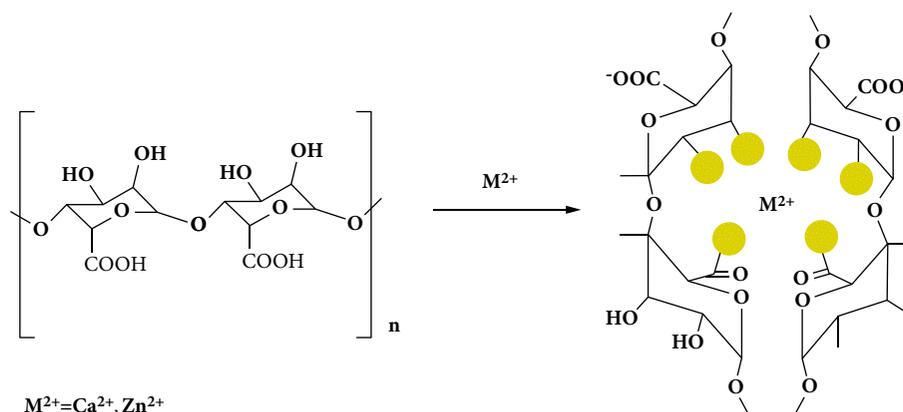


FIGURE 1: The “egg-box” model and the yellow circles represent the oxygen atoms involved in the coordination of the  $M^{2+}$ .

extraction time, and temperature are the main variables. Thus, the DE of pectin and extraction conditions are strongly coupled [13–17]. Nonetheless, few studies focused on influence of acidity and the hydrophilicity of extracting agents on the DE of pectin. Therefore, it is worth to study the relationships between the extraction conditions and DE of pectin.

The objective of this study was to clarify the influences of acid types on the DE of pectin. Sulphuric acid (SA) and citric acid (CA) were selected as the representative of strong inorganic acid and weak organic acid, respectively, to understand the influence of extraction agents. In addition, the response surface methodology (RSM) was employed to study the synergistic effect of extraction conditions (pH value, extraction time, and temperature) on DE. What is more, the effects of extraction conditions on the yield, monosaccharide composition, content of protein, and ferulic acid of pectin were studied.

## 2. Materials and Methods

**2.1. Materials.** The SBP was obtained from LÜ Yuan Sugar Industry Co., Ltd. (Hejing, Xinjiang Uygur Autonomous Region, China). The SBP was washed with tap water until the cleaning mixture became colorless. Finally, the washed SBP was dried in an oven at 105°C. The sulphuric acid and citric acid were purchased from Tianjin Fuguang Chemical Reagent Co., Ltd. (Tianjin, China). The *m*-hydroxydiphenyl, D-glucose, absolute ethanol, and sodium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals were of analytical grade and used without any treatment.

**2.2. Extraction of Pectin from Sugar Beet Pulp.** The pectin extraction process was followed the conventional thermal extraction, and the parameter values were determined based on our previous study. Briefly, the pH value of SA and CA solution were adjusted to 1, 2, and 3, respectively. Then, the pretreated SBP was added into the prepared solution according to the solid to liquid ratio (1:15) at different extraction temperature (80, 90, and 100°C) for preset extraction time (1, 1.5, and 2 h). The extract solution was then filtrated after adding two times volume of absolute ethanol, when the solution cooled

to the room temperature. Then the mixture was separated by centrifuging with 5000 rpm for 10 min. Finally, the pectin samples were washed with absolute ethanol and dried to constant weight at 40°C.

The characterization of pectin extracted at different conditions was in agreement with our published data [18]. Briefly, the total carbohydrate (Total CH) was determined by phenol-sulfuric acid photometric method, using D-glucose as standard [19]. The *m*-hydroxydiphenyl method was used for the determination of GalA content and D-galacturonic acid was employed as standard [20]. In addition, the aldonitrile acetate precolumn-derivatization gas chromatography method was employed to determine the monosaccharide composition [21]. The determination of content of the protein was followed to Lowry, Rosebrough, Farr, and Randall [22], using BSA as standard. The content of ferulic acid was determined using the colorimetric method at 320 nm, as referred to in the previous work [23, 24].

**2.3. Determination of DE.** The DE of pectin extracted from sugar beet pulp by CA and SA were determined according to the method by J. Singthong, S. Cui, W. S. Ningsanond, and H. D. Goff [25]. Briefly, dried pectin samples (0.2 g) were placed in a weighing bottle for titration and soaked with ethanol. Distilled water (20 mL) was added to dissolve the samples with stirring for 2 h at 40°C. The obtained solution was titrated with 0.1 mol/L NaOH in the presence of phenolphthalein. The results were the initial titre ( $I_t$ ). Then, the NaOH solution (0.1 mol/L, 10 mL) was added into the neutralized samples. The saponification process was carried out for 2 h at room temperature. Then, HCl solution (0.1 mol/L, 10 mL) was added. The NaOH (0.1 mol/L) was employed to titrate the excess HCl (0.1 mol/L). The amount of esterified carboxy groups of pectin was calculated from the volume of NaOH solution, which was the final titre ( $F_t$ ). The DE of pectin samples were calculated with,

$$DE (\%) = \frac{F_t}{F_t + I_t} \quad (1)$$

The DE of pectin extracted from SBP in the presence of SA and CA were marked DEs and DEc, respectively.

TABLE 1: Variable levels and the response of DE of pectin based on pH value, extraction temperature, and time.

Std.	Variable levels			DE (%)	
	$X_1$ pH value	$X_2$ Extraction temperature (°C)	$X_3$ Extraction time (h)	DEc	DEs
1	1	90	2	26.98	28.13
2	2	90	1.5	33.97	34.13
3	2	90	1.5	33.97	34.13
4	1	80	1.5	33.46	38.01
5	2	90	1.5	33.97	34.13
6	1	90	1	40.35	39.95
7	2	90	1.5	33.97	34.13
8	2	80	1	42.61	43.02
9	2	100	2	23.56	28.32
10	2	100	1	41.73	38.88
11	3	90	1	43.78	41.44
12	3	80	1.5	37.94	41.56
13	2	90	1.5	33.97	34.13
14	3	100	1.5	31.97	33.95
15	3	90	2	31.4	31.3
16	2	80	2	32.08	34.1
17	1	100	1.5	30.64	32.16

### 3. Results and Discussion

**3.1. Response Measurements.** In the present study, the three levels, three factors Box–Behnken experimental design was used to study the combined influences of independent variables on the DE of pectin extracted by SA and CA. The experimental parameters and the corresponding results are shown in Table 1.

As shown in Table 1, all the DE values are lower than 50%. These results indicated that the low-ester pectin samples were obtained. The DEc values range from 26.98% to 43.78%, and the values of DEs are in the range of 28.13%–43.02%. It is clear that the values of DEs are generally higher than those of DEc. The fact may be caused by the hydrolysis reaction of carboxylic acid ester in the acidic solution [26]. It is well known that the acid could provide the proton to oxygen atom of carboxyl group and positive electricity of carbonyl carbon atoms improves as a result. Then water play the role of the nucleophile to attack the carbonyl carbon in the acid-catalyzed hydrolysis reaction [27, 28]. Due to the fact that the CA is ternary weak acid, it had the competence to provide more protons than SA under the same pH condition. Thus, the weak polyacids is contributed to improve the hydrolysis reaction of methyl ester than the strong acid with same pH value. In addition, the adsorption process of acid molecule on the raw materials was a vital step in the acid-catalyzed hydrolysis reaction [29, 30]. From this perspective, the chemical constitution of CA was propitious to enrich on the surface of SBP, which mainly consist by the polysaccharides. Additionally, the reverse hydrolysis reaction was more likely to occur in strong acid solutions than in weak acid solutions [31, 32].

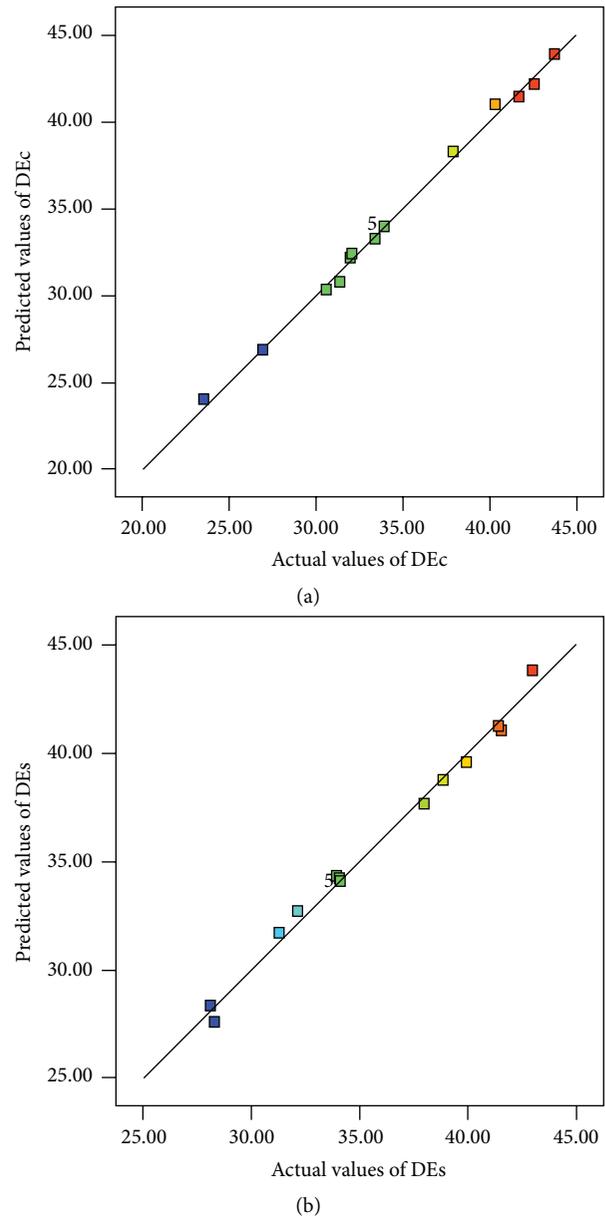


FIGURE 2: Comparison between predicted and observed DE.

**3.2. Estimated Model.** Box–Behnken experimental design was employed to estimate the correlation between the independent variables and the DE of pectin. The fitting models exhibited by

$$\begin{aligned} \text{DEc} = & -22.86750 + 7.72250X_1 + 1.49312X_2 \\ & + 0.87750X_3 - 0.07875X_1X_2 + 0.495X_1X_3 \\ & - 0.382X_2X_3 + 0.0825X_1^2 - 5.5 \times 10^{-3} \times X_2^2 + 6.3X_3^2, \end{aligned} \quad (2)$$

$$\begin{aligned} \text{DEs} = & 191.3225 + 1.12X_1 - 2.92975X_2 - 9.07X_3 \\ & - 0.044X_1X_2 + 0.84X_1X_3 - 0.082X_2X_3 \\ & + 0.7075X_1^2 + 0.015825X_2^2 + 1.47X_3^2. \end{aligned} \quad (3)$$

The predicted values of DEc and DEs were calculated by the regression model and compared with experimental values. The

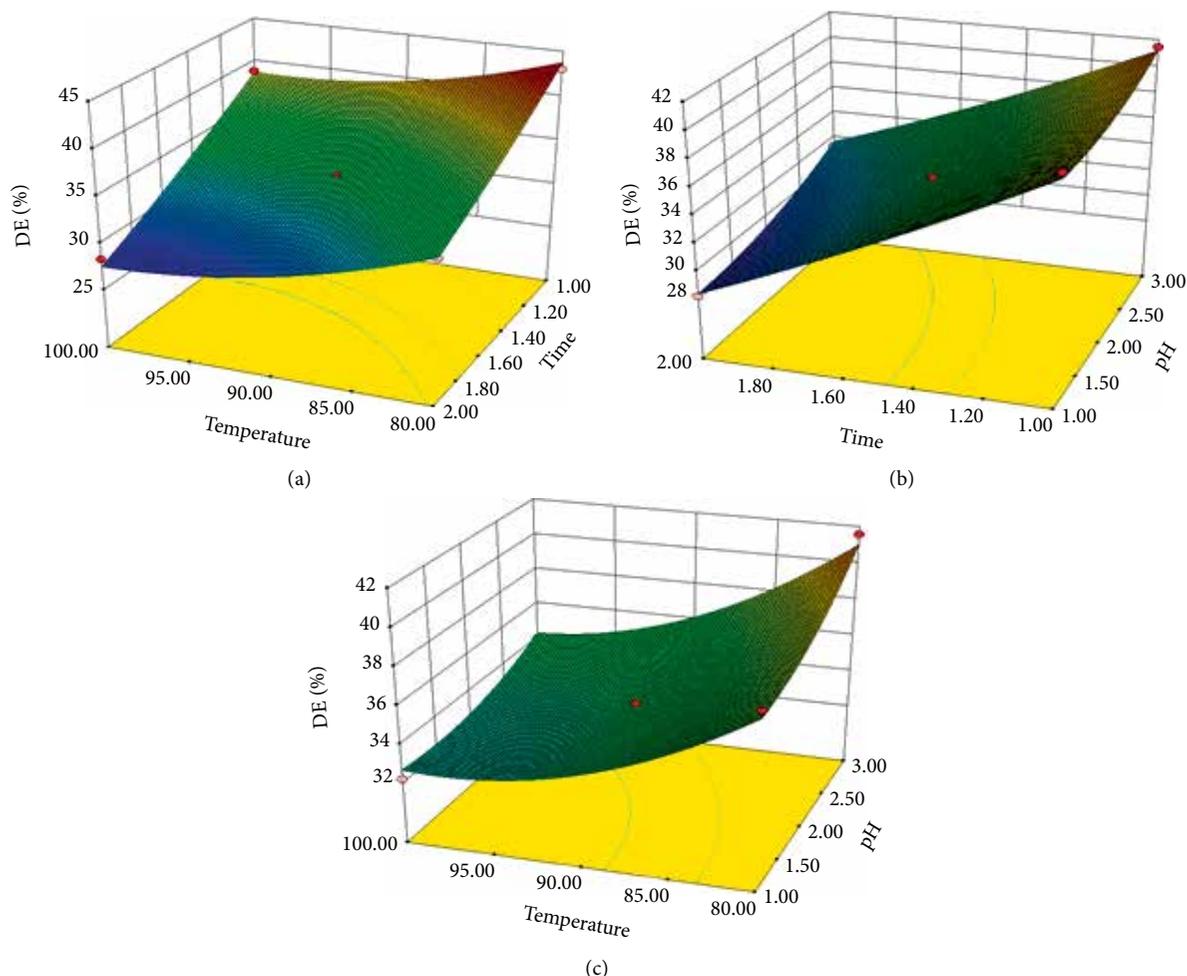


FIGURE 3: Surface plots for DE of pectin extracted by sulphuric acid. (a) Effect of extraction time and temperature on the DEs with pH of 2. (b) Effect of extraction time and pH on the DEs with extraction temperature of 90°C. (c) Effect of extraction temperature and pH on the DEs with extraction time of 1.5 h.

results are shown in Figures 2(a) and 2(b). The total determination coefficient ( $R^2$ ) of DEc and DEs are 0.996 and 0.992, respectively, indicating reasonable fit of the models to the experimental data [33].

The analysis of variance (ANOVA) was employed to statistical significance of the two models, and the corresponding results are listed in Tables 2 and 3. Generally, higher  $F$ -value and lower  $P$ -value indicate higher significance of the independent variables and the models [26, 34, 35]. The  $P$ -values of the two models are all less than 0.0001, which shows the high significance of the models [16, 26, 35]. No matter which acid was used, the extraction time, temperature, and pH value significantly influenced the DE of extracted pectin by analysing  $P$ -value and  $F$ -value of the liner terms. Moreover, the  $P$ -values of interaction in the CA extraction process were all higher than that of SA extraction process. The results indicated that the stronger interaction of the variables in the citric extraction process, especially for the interaction between the extraction temperature and pH value. It can be explained by the fact that the weak acid present better ionization property at higher temperature conditions.

**3.3. Analysis of Response Surface.** The relationship between DE and experimental variables are shown with the 3D representations of the response surfaces in Figures 3 and 4. It is clear that the DE decreased with the increasing of extraction temperature and pH value, and the lapse of extraction time. The trend is in agreement with the previous work [4, 13, 14]. As can be seen in Figures 3 and 4, the variation trend of DEc conforms with DEs with the alteration of independent variables. To summarize, the increasing of extraction temperature, time, and concentration of acid contributes to the low-ester pectin [36]. Figure 3(c) shows that the DEs changed more obviously at high extraction temperature than that at low extraction temperatures. The phenomena appeared that relatively lower temperatures contributed to the hydrolysis of the carboxylic acid ester in SA solution. On the contrary, the DEc increases sharply at low extraction temperature which is unfolded in Figure 4(c). By comparing the two plots, the variation in the CA extraction process occurred more acutely. The results indicated that organic polyatomic acid was propitious to hydrolysis reaction at the same pH condition. In addition, the influence of extraction temperature on the DE was lower

TABLE 2: Results of analysis of variance (ANOVA) for DEC.

Source	Sum of squares	DF	Mean square	F-value	P-value
$X_1$	23.32	1	23.32	98.68	<0.0001
$X_2$	41.36	1	41.36	174.98	<0.0001
$X_3$	370.60	1	370.60	1567.94	<0.0001
$X_1X_2$	2.48	1	2.48	10.50	0.0143
$X_1X_3$	0.25	1	0.25	1.04	0.3425
$X_2X_3$	14.59	1	14.59	61.74	0.0001
$X_1^2$	0.029	1	0.029	0.12	0.7379
$X_2^2$	1.27	1	1.27	5.39	0.0533
$X_3^2$	10.44	1	10.44	44.19	0.0003
Model	464.05	9	51.56	218.15	<0.0001
Residual	1.65	7	0.24	—	—
Lack of fit	1.65	3	0.55	—	—
Pure error	0.000	4	0.000	—	—
Cor total	465.71	16	—	—	—

TABLE 3: Results of analysis of variance (ANOVA) for DEs.

Source	Sum of squares	DF	Mean square	F-value	P-value
$X_1$	12.50	1	12.50	36.56	0.0005
$X_2$	68.33	1	68.33	199.87	<0.0001
$X_3$	214.66	1	214.66	627.91	<0.0001
$X_1X_2$	0.77	1	0.77	2.27	0.1760
$X_1X_3$	0.71	1	0.71	2.06	0.1940
$X_2X_3$	0.67	1	0.67	1.97	0.2035
$X_1^2$	2.11	1	2.11	6.17	0.0420
$X_2^2$	10.54	1	10.54	30.84	0.0009
$X_3^2$	0.57	1	0.57	1.66	0.2381
Model	311.86	9	34.65	101.36	<0.0001
Residual	2.39	7	0.34	—	—
Lack of fit	2.39	3	0.80	—	—
Pure Error	0.000	4	0.000	—	—
Cor Total	314.25	16	—	—	—

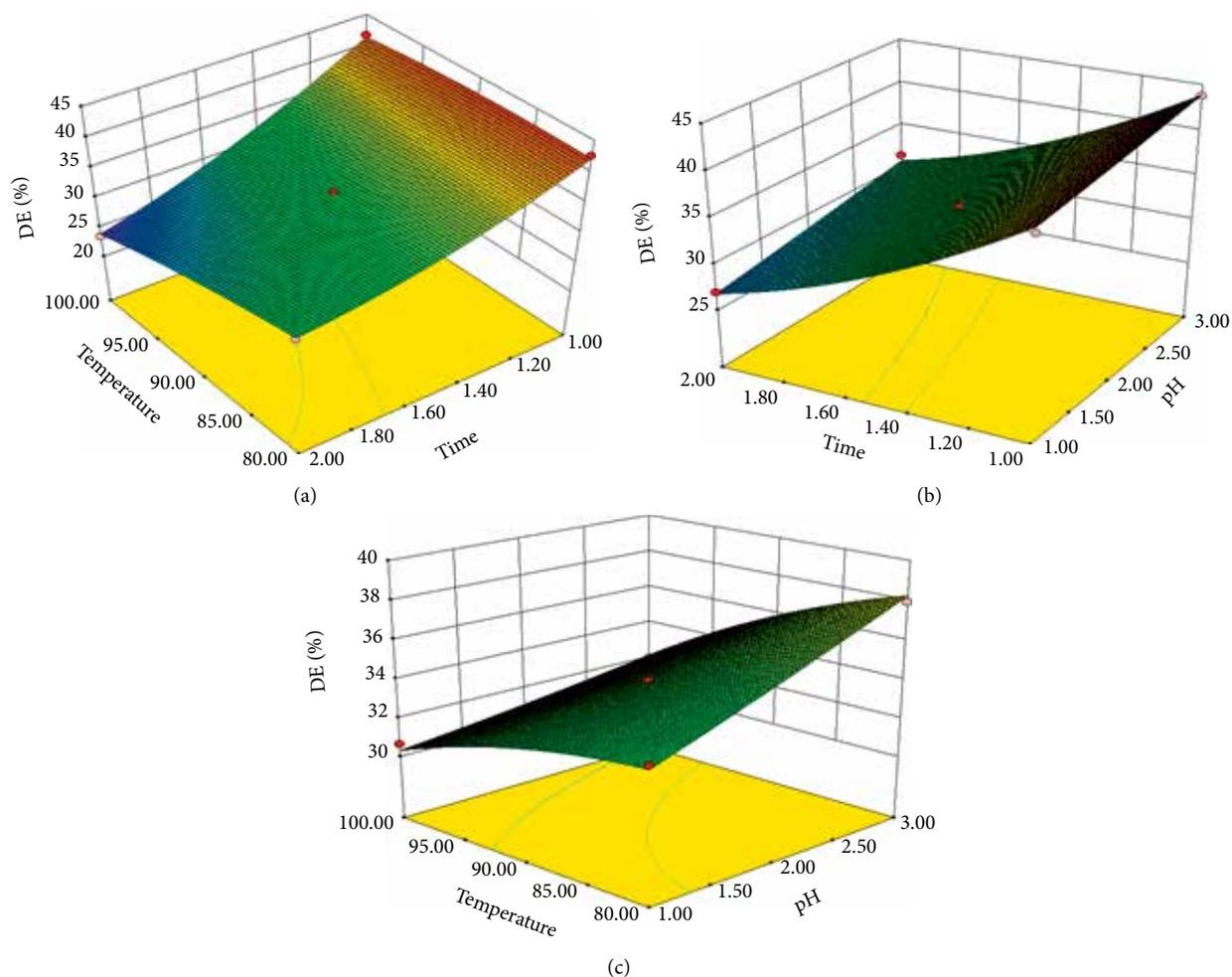


FIGURE 4: Surface plots for DE of pectin extracted by citric acid. (a) Effect of extraction time and temperature on the DEc with pH of 2. (b) Effect of extraction time and pH on the DEc with extraction temperature of 90°C. (c) Effect of extraction temperature and pH on the DEc with extraction time of 1.5 h.

TABLE 4: Yield and chemical composition of pectins extracted by SA and CA from sugar beet pulp (%).

Std.*	Variable levels			Acid	Yield	Ferulic acid	Protein	Responses					
	$X_1$	$X_2$	$X_3$					Total CH	GalA	Rha	Ara	Glc	Gal
1	1	90°C	2h	SA	21.08	0.73	1.87	76.3	74.2	0.4	6.3	0.8	4.1
				CA	18.25	0.86	1.69	79.6	66.5	0.4	4.8	0.4	1.9
17	1	100°C	1.5 h	SA	22.24	0.81	1.56	75.2	72.1	0.3	5.9	0.7	3.7
				CA	17.52	0.74	1.96	77.3	67.1	0.3	4.2	0.3	1.7
12	3	80°C	1.5h	SA	5.23	1.37	2.76	78.2	66.3	3.4	11.3	1.3	9.1
				CA	6.79	1.26	3.09	80.1	62.6	3.1	9.6	0.8	6.8
15	3	90°C	2h	SA	6.12	1.12	3.01	78.6	69.1	3.1	11.1	1.5	8.8
				CA	8.81	1.42	2.63	79.5	63.7	2.7	9.1	0.6	6.5

\*The Std. and variables are corresponding to the Table 1.

than that of time and pH in the SA extraction process, while it was opposite in the CA extraction process.

**3.4. Yield and Chemical Composition.** The influence of variables on the yield of pectin was an important aspect in the pectin extraction process at one time. Abundant studies have focused on the investigation of effect of variables on the yield, and the hydrolysis of glycosidic bond has been considered to be the major reaction in the presence of acid [37]. Thus, we showed incomplete yield values of pectin extracted in the two extreme conditions (pH = 1 and pH = 3) in Table 4. It is evident that the lower pH value contributed to higher extraction yields. Additionally, weak polyacid contributed to higher yields in higher pH values than the strong acid due to the fact that the weak polyacid could constantly provide more protons. The content of protein was also determined, and the values was in the range of 1.56%–3.09%. The content of ferulic acid ranges from 0.73% to 1.42%.

The monosaccharide composition of pectin extracted by SA and CA are shown in Table 4. The content of Total CH and GalA of pectin extracted by SA were higher than that of pectin extracted by CA. In addition, the content of monosaccharide shows an opposite trend. In general, the lower pH and longer extraction time contributed to the lower content of monosaccharide. The results are in agreement with the previous works [18, 38, 39] and may be caused by the hydrolysis reaction between side chain and main chain of pectin [40–44].

## 4. Conclusions

The CA and SA solution were employed to extract pectin from SBP. The influence of variables including pH value, extraction time, and temperature on the DE were studied by RSM. The obtained pectin samples were all low-ester pectin. The DE value of the pectin extracted by CA and SA ranged from 26.98% to 43.78% and 28.13% to 43.02%, respectively. The DE value decreased with the increasing of extraction temperature, expanding of extraction time, and decreasing of extraction pH. The ANOVA indicated that the DE of pectin was significantly influenced by the variables including pH, extraction time, and temperature. What is more, the stronger interaction of the variables existed in the CA extraction process. The

content of protein and ferulic acid are in the range of 1.56%–3.09% and 0.73%–1.42%, respectively. The pectin extracted by SA present higher content of Total CH and GalA than that of pectin extracted by CA. The content of monosaccharide is just opposite.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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