OPTIMIZATION OF THE PECTIN EXTRACTION FROM POMELO PEELS BY OXALIC ACID AND MICROWAVE

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Abstract. The objective of research was the study on main factors (pH, irradiation time, the rate of solvent/dried peels of oxalic acid and using microwave) affecting the productivity of crude pectin extraction from pomelo peels. Optimizing the factors was used the Response Surface Methodology (RSM) with Central Composite Face Centered (CCF) model. There was the interaction between rate of solvent/dried peels, pH and irradiation time with amount of pectin crude. The optimal results were crude pectin 18.58% with pH=4.22 and rate of solvent/dried peels (v/w) was 36.05/1 and irradiation time during 8.5 minutes at 660W of real power of microwave. Obtained crude pectin was light white color and pure pectin was approximate 91%. It can use widely in the food industry, for instance additive, jam, beverage.

Key words: crude, irradiation time, pH, response surface, viscosity, yield.

Introduction

During the renovation period, food additives are one of the leading trends to make quality products with high safety and good sensory evaluation.

Pectin is widely used as gelling agent and stabilizer in a variety of food, pharmaceutical, cosmetic products [THAKUR et al., 1997].

Pomelos has a scientific name is *Citrus Maxima* in *Citrus* group and Rutaceace. Trees are planted widely distributed from North to South Vietnam with many different varieties.

The peels of pomelo (spongy white peel) represent about 30% of the total fruit weight; it was a potential source of pectin [PAWADEE et al., 2013].

Pectin is a family of complex polysaccharides of galacturonic acid present in primary cell wall and middle lamella of plant tissues.

Pectin was usually extracted from several resources, such as orange peel, apple pomace, lemon and sugar beet pulp by inorganic acid such as HCl [KANSCI et al., 2008] or HNO$_3$ [CARMEN et al., 2011], and organic acid such as citric acid [YAN et al., 2012], but they almost don't use oxalic acid in pectin industry.

Oxalic acid used widely chemical and food industry, especially in postharvest technology [YUEMING et al., 2013].

In this case, we combined oxalic acid with microwave to extract pectin with high productivity.

Because microwave assisted extraction (MAE) is the new method for irradiation some good substance from raw material it has a lot of advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost.

Compared with another method, apparatus of microwave assisted extraction is simpler and cheaper [HAO et al., 2002].

MAE increased capillary porous characteristics and water absorption capacity of plant material.

These changes provide an opportunity for improving the extraction yield of target analytes from plant material [KRATCHANOVA, 2004].

Response surface methodology (RSM) using Central Composite Face Centered (CCF) design was employed in this research.

There are various advantages in using statistical methodologies in terms of rapid and reliable short listing of process

conditions, understanding interactions among them and tremendous reduction in total number of experiments [MARY *et al.*, 2003].

For this reason, it is necessary to optimize the pectin extraction to improve yield and quality.

Currently, we use the design and statistical approaches of response surface methodology to optimize some factors of oxalic acid to improve pectin yield.

Material and methods

Materials

Ripe Nam Roi pomelo was chosen as the raw material in these experiments. It was harvest from Tien Giang (Vietnam).

The peels were removed with a paring knife, cut into small pieces and then were blanched in hot vapor water for 10 min.

After that, it were dried with hot air at 70°C, crushed and screened by sieve, ensure particles smaller than 0.6 mm in diameter were selected.

Pectin extraction

10g of pomelo peels were soaked in oxalic acid solution 0.25% (v/v) with rate peel/acid from 1/29 to 1/49 and pH range of 4.2–5 at 30°C.

Then it is placed in the microwave, during 6–12 min at level 660W.

After microwave heating, the mixture was cooled down to room temperature and filtered using filter paper.

Precipitated period was modified against method of Farzin [FARZIN *et al.*, 2011], adding 96% ethanol with the rate pectin solution/alcohol was 1/3 during 60 minutes and the coagulated pectin was separated by centrifuge and washed twice with 96% ethanol to remove the mono and disaccharides.

It was dried at 70°C for 8h in an oven and was stored in bags.

Determination of pectin yield

Pectin yield was calculated from equation bellows

$$Y = \frac{m_o}{m} \cdot 100$$

– m_0 (g) is the weight of dried pectin
– m (g) is the weight of dried pomelo peels

Determination of pure pectin

According to MUI (2001), crude pectin (0.15g) was added in 250 mL flask, then adding 100mL of 0.1N NaOH.

Crude pectin was soaked in NaOH solution during 7 h., we add 50 mL of 1N CH_3COOH and 50 mL $CaCl_2$ after 5 minutes, keep it in 1 h.

The solution was boiled in 5 minutes, filtered by filter paper and dried about 1 hour.

Calcium pectate was washed by hot water until not have ion Cl^- in solution and dried it during about 2 hour at 105°C.

The pure pectin (%) was calculated according to the following formula below:

$$P(\%) = \frac{m \times 0{,}92 \times 100}{M}$$

– m (g): weight of calcium pectate
– M (g): weight of crude pectin
– 0.92: pectins have 92% in calcium pectate

Determination of degree of esterification (DE)

This method was slight modified from titrimetric method of Pinherio [PINHERIO *et al.*, 2008].

Pectin (0.5 g) was added in 250 mL flask and dissolved in 5 mL ethanol, 1 g NaCl and one or two phenolppthalein.

Adding 100 mL of warm deionized water dissolved pectin.

The solutions were then titrated with 0.1N NaOH and the results were recorded as V_1.

Then 25 mL of 0.25 N NaOH was added and the solutions were stirred at room temperature for 3 minutes.

After that, 25 mL of 0.25N HCl was added and the solutions were shaken until the pink color disappeared.

The solutions were titrated with 0.1N NaOH and the final results were recorded as V_2.

The DE was calculated according to the following formula below:

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

Determination of viscosity by Rheometer (Brookfield DV–III Ultra–USA), spindle "61" and speed 100r/min

Experimental design and statistical analysis

Response surface methodology (RSM) was used to determine optimum conditions for pectin production by software Modde 5.0.

There are three factors including pH (x_1), the rate of solvent/dried peels (x_2) and irradiation time (x_3) (Table 1).

It can affect to target retrieval performance crude pectin (y) were determined using optimization method. Influence of factors to target function was described according to equation below:

$$y = b_o + \sum_{i=1}^{n} b_i\, x_i + \left(\sum_{i=1}^{n} b_{ii} x_i\right)^2 + \sum_{i<j}^{n} b_{ij} x_i x_j.$$

In this study, n–value was 3 so equation (1) can be written:

$$y = b_o + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1{}^2 + b_{22} x_2{}^2 + b_{33} x_3{}^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3$$

Table 1.

Codes and actual levels of the in dependent variables for design of experiment

Independent variables	Symbols	Coded levels		
		−1	0	1
pH	x_1	4.2	4.6	5.0
The rate of solvent/dried peels (v/w)	x_2	29/1	39/1	49/1
Irradiation time (minute)	x_3	6	9	12

Using model in this case is Central Composite Face (CCF) (Table 2).

The star points are at center of each face of factorial space, so α=±1.

This variety requires 3 levels of each factor.

CCF designs provide relatively high quality predictions over the entire design space and do not require using points outside original factor range, require 3 levels for each factor [MARY *et al.*, 2003].

Table 2.

Matrix layout experiments and results

Run No	Coded levels			Real values			Yield of crude pectin (%)	
	x_1	x_2	x_3	x_1	x_2	x_3	Observed	Predicted
1	13	−1	−1	4.2	29/1	6	17.42	17.5356
2	1	−1	−1	5.0	29/1	6	13.27	12.8995
3	−1	1	−1	4.2	49/1	6	16.06	15.9465
4	1	1	−1	5.0	49/1	6	11.29	11.6005
5	−1	−1	1	4.2	29/1	12	17.03	16.8366
6	1	−1	1	5.0	29/1	12	11.02	11.2505
7	−1	1	1	4.2	49/1	12	15.32	15.8076
8	1	1	1	5.0	49/1	12	10.51	10.5115
9	−1	0	0	4.2	39/1	9	18.81	18.5138
10	1	0	0	5.0	39/1	9	13.72	13.5478
11	0	−1	0	4.6	29/1	9	13.57	13.7878
12	0	1	0	4.6	49/1	9	13.31	12.6238
13	0	0	−1	4.6	39/1	6	13.94	13.9978
14	0	0	1	4.6	39/1	12	13.63	13.1038
15	0	0	0	4.6	39/1	9	14.21	14.3695
16	0	0	0	4.6	39/1	9	14.22	14.3695
17	0	0	0	4.6	39/1	9	14.21	14.3695
18	0	0	0	4.6	39/1	9	14.21	14.3695
19	0	0	0	4.6	39/1	9	14.21	14.3695
20	0	0	0	4.6	39/1	9	14.22	14.3695

( ): Running the software Modde version 5.0 to predict obtained model

Result and discussion

Pectin was extracted from pomelo peels, which ranged from 10.51% to 18.81%.

The highest yield was achieved when extraction conditions were pH 4.2, the rate of solvent/dried peels 39/1 (v/w) and irradiation time 9 min at 660W.

This power of microwave are in accordance with studied reported by Farzin [FARZIN *et al.,* 2011], they have highest yield at 630W.

Table 3.

Analysis of variance (ANOVA) for the fitted quadratic polynomial model for pectin production

y	DF	SS	MS	F	p	SD
Total	20	4117.13	205.856			
Constant	1	4037.91	4037.91			
Total Corrected	19	79.2148	4.1692			2.04186
Regression	9	77.5644	8.61827	52.2187	0.000	2.93569
Residual	10	1.65042	1.65042			0.406253
Lack of Fit	5	1.65029	0.330057	12375.7	0.000	0.574506
Pure Error	5	0.00013	2.666e–005			0.005128

Through Anova table (Table 3) found $p_{value}=0.000<0.05$: there are differences between the experiments and compatible with experiment. So we can write regression equation below.

Table 4.

Results of regression analysis of the full factorial model

y	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	14.3695	0.13996	1.84307e–016	0.311179
x_1	−2.483	0.128469	2.99717e–009	0.286243
x_2	−0.582002	0.128469	0.00109089	0.286243
x_3	−0.446999	0.128469	0.00592672	0.286243
$x_1.x_1$	1.66136	0.24498	4.84907e–005	0.545845
$x_2.x_2$	−1.16364	0.24498	0.00078033	0.545845
$x_3.x_3$	−0.818636	0.24498	0.00746976	0.545845
$x_1.x_2$	0.0724998	0.143632	**0.624664**	0.32003
$x_1.x_3$	−0.2375	0.143632	**0.129227**	0.32003
$x_2.x_3$	0.14	0.143632	**0.352692**	0.32003
N=20	Q^2 =	0.779	Cond. no. =	4.5044
DF=10	R^2 =	0.979	Y – miss =	0
	$R^2_{Adj.}$ =	0.960	RSD =	0.4063
			Conf. lev. =	0.95

Table 4 showed the regression coefficients of the response function.

The coefficients $x_1.x_2$, $x_1.x_3$ and $x_2.x_3$ should be removed from the equation, do not interact together because it were not significant (p>0.05).

And showed that pH, the rate of solvent/dried peels, irradiation time were completely independent of each other and strong influence on retrieval performance pectin.

Derive regression equations showing the dependence of pectin–based retrieval performance on three factors mentioned above:

Through Anova table (Table 3) found $p_{value}=0.000<0.05$: there are differences between the experiments and compatible with the experiment. So we can write the regression equation below.

$$y = 14.3695–2.483x_1–0.582x_2–0.4469x_3+1.6613x_1^2–1.1636x_2^2–0.8186x_3^2.$$

Table 4 showed the experimental yields fitted the polynomial equation of the first degree well as indicated by high R^2 (Coefficient of determination) value was 0.979. The R^2_{adj} was 0.960 and the Q^2 was 0.779, which indicates that the model is good. For a good statistical model, the R^2 value should be in the range of 0–1.0, and the nearer to 1.0 the value is, the more fit the model is deemed to be; predictive ability of model manifest by Q^2 with fail–safety achieved R^2. According to Eriksson [ERIKSSON *et al.,* 2008], $Q^2> 0.5$ and

R^2–Q^2<0.2–0.3 for equations with empirical sense.

So the model is accurate acquisition and the ability to apply in practice to provide data with high accuracy.

The linear regression coefficient for pH, the rate of solvent/dried peels and irradiation time were negative, indicating higher pectin yield at lower these factors. In summary, lower these factors improved pectin yield, independent of each other.

The quadratic regression coefficient for pH was positive and rate of solvent/dried peels and irradiation time were negative, it affected strongly to pectin yield.

Hence, pH increases with decreasing extraction rate of solvent/dried peels, irradiation time and vice versa.

Based on our model have been factors in the prediction equations to obtain the maximum performance.

Table 5.

Results of optimal conditions

Results	pH (x_{1opt})	Rate of solvent/dried peels (v/w) (x_{2opt})	Irradiation time (x_{3opt})	% Crude pectin (y_{max})
Predictive model	4.22	36.05	8.54	18.6249
Experimentation	4.22	36.05	8.54	18.58±0.32

In table 5, we conduct empirical test, the result is the difference between the retrieval performances from empirical lower than 0.045% predicted model.

Therefore, model is set up correctly and has ability to predict quite high, so application can be put into practice. The figure 1 and figure 2 show the pectin yield that depends on pH, rate of solvent/dried peels and irradiation time.

Optimal area has border value 18.31%, was covered by rate of solvent/dried peels (x_2=35/1–37.5/1) (v/w) and irradiation time (x_3=8–9 min) at pH (x_{1opt}=4.22).
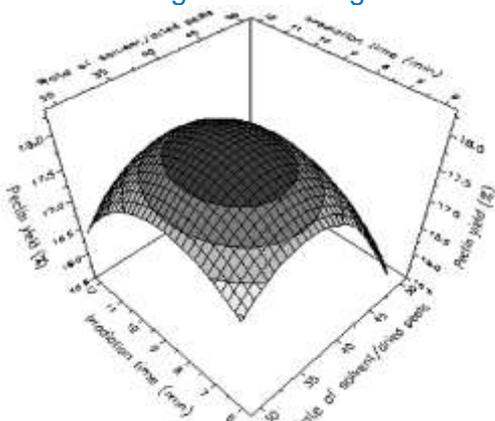

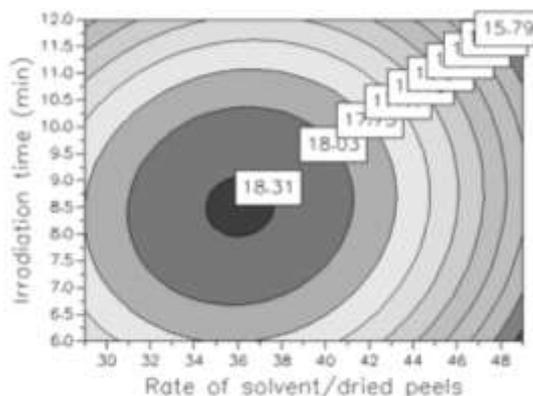**Figure 1.** Response surface plot of pectin yield at x_{1opt}=4.22

Figure 2. Prediction plot of pectin yield at x_{1opt}=4.22

Irradiation time is very important parameter affecting the extraction yield; it is the essential factor to choose appropriate irradiation time to obtain the maximum extraction yield of pectin.

Dried peels was absorbed the energy from microwave promoted the thermal accumulation of the extraction and dissolved pectin in solution, if we extend irradiation time, pectin will be degraded and the extraction yield decrease [XIANZHE *et al.,* 2011].

In this case, pH also affects to extraction yield, pectin extraction at pH 4.22 gives the highest yield, oxalic acid can contact and dissolve protopectin from peels into soluble pectin [EL–NAWAWI and SHEHATA, 1988] like hydrochloric, sulfuric or nitric acid augmenting the pectin yield. In addition, rate of solvent/dried peels was also the main factor to evaluate yield of pectin, the suitable rate make the highest extraction yield, the cell walls were ruptured, which resulted in easy release

of pectin into the surrounding environment [SIVAKUMAR *et al.,* 2013].

Based on the data received from table 2, we get the graph shows the correlation between the pectin yield obtained from the experimental and model predicted.
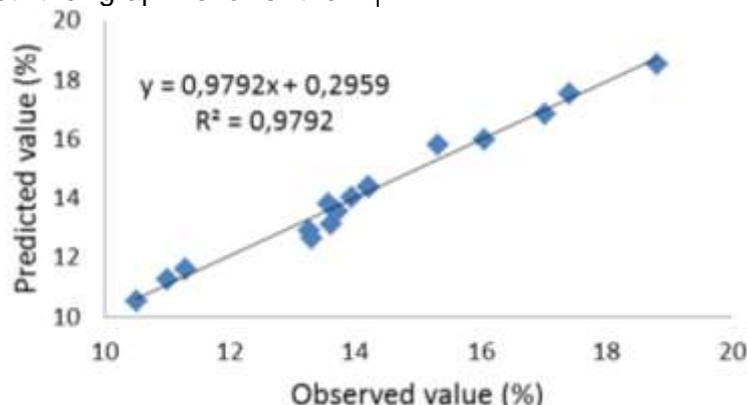

Figure 3. Plot showing the distribution of experimental versus predicted values

In addition to determination coefficients, the adequacy of the model was evaluated through various diagnostic plots such as predicted versus actual.

The predicted values obtained from the developed model were quite close to the experimental values and lie reasonably close to the straight line and indicated the adequate agreement with real data. In figure 3, $R^2=0.979$, it means that the equation can explain 97.9% of the actual data and this model could not explain only 2.1% of the overall effects.

Consequently, the results between predicted value and observed value had closely linear correlation.

In figure 4, viscosity of crude pectin was quite low (<20cp) with some concentration solutions 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v), they were Newtonian fluid [VORAGEN *et al.,* 1995]. Using calcium pectate method tests the crude pectin samples, the results shows that pure pectin obtains approximate 91% and it has DE=91.83% (High Methoxyl Pectin, DE>50%).

This result was higher than another study of Pawadee [PAWADEE *et al.,* 2014] (Using HCl and HNO_3 extracted pectin from pomelo peels, DE=59.4–70.7% and crude pectin yield=8.32–24.26%) and crude pectin products were in figure 5.
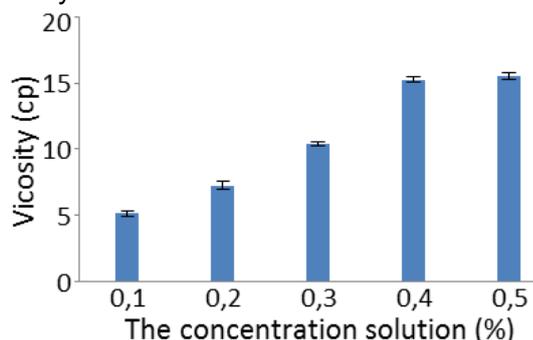

Figure 4. Viscosity of crude pectin

Figure 5. Pectin product

Conclusion

Oxalic acid and microwave have great influence on the performance recovered pectin.

The experimental performance obtained is maximum 18.56% with pH=4.22, the rate of solvent/dried peels=36.05/1 and irradiation time 8.5 min at 660W in microwave.

Using the optimal method of target function, we exposed regression equation and this equation can be applied on actual model:

$$y=14.3695–2.483x_1–0.582x_2–0.4469x_3+1.6613x_1^2–1.1636x_2^2–0.8186x_3^2.$$

These factors were absolutely independence, not interactive together.

This model had the high R^2, R^2_{adj} and Q^2 and was absolutely according to actual production. It was quite easy to select and modify some parameters in pectin processing.

Product was high methoxyl pectin (DE=91.83%) and achieves 91% pure pectin and low viscosity.

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