Optimization of Microwave Assisted Extraction of Pectin from Orange Peel Using Response Surface Methodology

Anil Kute¹, Debabandya Mohapatra²*, Bhushan Babu² and B.P.Sawant¹

¹College of Agricultural Engineering and Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani-431 402 (M.S.), India.
²ICAR-Central Institute of Agricultural Engineering, Nabi Bagh, Berasia Road, Bhopal-462038, India.

*Corresponding Author:  Debabandya Mohapatra  Email: debabandya@gmail.com

Received: 15/04/2014 Revised: 21/06/2015 Accepted: 23/06/2015

Abstract

Microwave assisted extraction of pectin from dried orange peel powder was carried out in a microwave oven with acidified water using concentrated nitric acid. Process parameters like pH of the acidified water (1.5, 2, 2.5), solvent: solid ratio (10, 20, 30), microwave power (360, 540, 720 W), and extraction time (30, 60, 90 s) were optimized by response surface methodology, using full factorial design. The optimized condition was found to be pH 1.5, solvent: solid ratio 20, microwave power of 630 W, extraction time of 89 sec for an optimized pectin yield of 13.32%. A second order polynomial equation generated for predicting pectin yield was validated by conducting 3 trials at the optimized point.

Keywords: Orange peel, Pectin, Microwave extraction, Pectin yield, Response surface methodology.

1. Introduction

Pectin, a polysaccharide has a varied use in food, cosmetic and pharmaceutical industries. It is sourced from plant cells, which may range from fruit peels and pomace to grain hull and cob heads. Though it can be extracted from various sources, the normal raw materials are apple pomace and citrus peels, from which pectin was obtained by acid extraction and precipitation using alcohols or aluminium salts (May, 1990).

Several methods are used for extraction of pectin from plant sources, such as conventional method of boiling in acidified water, ultrasonic assisted extraction (Bagherian et al., 2011), microwave assisted extraction (MAE) (Fishman et al., 1999; Liu et al., 2006; Yeoh et al., 2008), enzymatic extraction (Donaghy and McKay, 1994; Ptichkina et al., 2008), high hydrostatic pressure treatment (Guo et al., 2012; 2014) methods to name a few. The most widely used method is boiling of the pectin sources in acidified water, followed by coagulation with ethanol. However, this method is a time consuming and an energy guzzling process. On the other hand, microwave assisted extraction has shown potential in terms of saving time and energy as well as solvent consumption. The popularity of microwave energy has become many fold and microwave have been used in various thermal processing operations like thawing, puffing and popping, baking, blanching, drying and dehydration, pasteurization, sterilization, disinfections, extraction of pigments, essential oil, polyphenols, pectin (Sumnu et al., 2005; Zhang et al., 2006; Chandrasekaran et al., 2013; Routray and Orsat, 2012; Joshi et al., 2014; Mohapatra et al., 2014a,b; 2015; Mishra et al., 2015) to name a few. The versatility of the microwave systems has made it one of the favorite thermal processing methods. Its commercial adoption in the field of extraction has seen a constant growth worldwide, in the last few decades.

The principle of heating during MAE is based on dielectric heating of plant molecules through the exposure of microwaves. When microwaves pass through the plant tissue, the energy absorbed causes volumetric heating due to the dipole rotation of polar molecules like water, which causes vibration as there is molecular collision, leading to heat generation inside the plant tissue (Mohapatra and Mishra, 2011). When orange peels are subjected to microwave radiation, there is inactivation of pectin esterase enzyme and destruction of orange skin cells due to rapid heat generation in microwave environment (Zhongdong et al., 2006). Since the pectin esterase interacts with the pectic substances in the orange peels and reduces their solubility, their inactivation improves the pectin extraction. Moreover, due to the disintegration of parenchyma cells, there is also increase in specific surface area, which facilitates the water absorption capacity of the plant cell (Kratchanova et al., 2004).
Higher absorption of water molecule to the specific cite facilitates the extraction of bioactive compounds.

Considering all the merits of MAE of pectin from orange peels, in this investigation, microwave assisted extraction of pectin from orange peel was carried out in order to optimize the processing parameters through response surface methodology.

2. Material and Method

2.1 Preparation of Orange Peel Powder from Orange Peel

Oranges (5 kg) were obtained from the local market of Bhopal. Peels were removed from the fresh oranges and weighed in analytical balance to find out the weight percentage of the whole fruit. The orange peel was chopped by using knife, followed by drying in a tray drier (NSW, India) at 50 °C for 5 h with intermittent stirring. The dried peels were grinded in a domestic mixture grinder (Philips India limited, Kolkata) for 15 s to obtain powdered sample. Final moisture content of the powdered sample of the orange peel was estimated by hot air oven method and the powder was stored in sealed aluminum laminated pouch bags in a freezer till further experimentation.

2.2 Microwave Assisted Extraction of Pectin from Dried Orange Peel

Extraction of pectin was performed with the help of a domestic microwave oven (CE11111TL, Samsung, Thailand), at working frequency 2450 MHz and maximum power output 900W, with adjustable microwave power and irradiation time under different conditions. Orange peel powder (1g) was taken in a 100 mL Pyrex beaker and acidified water of different pH (1.5, 2, 2.5) was added to it for different solvent to solid ratio (10, 20 and 30 mL/g). The solution was placed in the middle of the microwave equipment over a rotating disc and exposed to different microwave power levels (80, 60, 40%, having corresponding microwave power level of 360, 540 and 720 W, respectively) and irradiation time (30, 60, and 90 s). The pH of the solvent was adjusted with the help of HNO₃ (1M). After the microwave treatment, the mixture in Pyrex beaker was allowed to cool down to room temperature. The sample was then centrifuged in a refrigerated centrifuge (RSMI G-12 plus, India) at 4 °C, 8000 rpm for 10 min (Wang et al., 2007).

The insoluble residue was recovered and the supernatant was precipitated with an equal volume of 95% (v/v) ethanol. The coagulated pectin mass was washed with 95% (v/v) ethanol for three times to remove the mono and disaccharides (Maran et al., 2013). The coagulated pectin was then subjected to freeze drying in a laboratory freeze dryer (OD-12, Delvac pumps, Pvt ltd, Chennai, India) at -40°C for 24 h. Samples were then weighed and the pectin yield was calculated. The pectin was milled in an analytical mill and packed in aluminum laminated pouches and sealed. The sealed packs were stored at 6 °C in a refrigerator till further analysis.

The pectin yield (PY) was calculated by weighing the pectin obtained and was expressed in percentage with respect to the orange peel powder taken for extraction.

2.3 Design of Experiment and Process Optimization

Response surface methodology was employed to optimize the process parameters. The method of response surface methodology deals with the problem of seeking the condition of an experiment, which are optimal or most desirable. To optimize process parameter pectin extraction for 3 level 4 full factorial design were used and the optimization of process parameter were done using Design Expert 9.0.1 (Statease, USA, Trial version). Based on literature and preliminary experiments, independent variable pH (A), solvent:solid ratio (B), microwave power (C), and time (D) were considered as important factors impacting the yield of end product. All these variable were closely monitored and accurately measured during experimentation. The sole response pectin yield (Yk) was assumed to be a function of the dependent variables f1(1, 2, 3, 4):

\[ Y_k = f(k, A, B, C, D) \]

Mean values of the full factorial experiments were considered for the optimization study by response surface methodology. A total of 87 experiments, including 6 runs at the central point were conducted. A quadratic polynomial equation was used to approximate the function \( f_k \) using response surface methodology (RSM).

\[ Y_k = b_{0} + \sum b_{ki}X_i + \sum b_{kii}X_i^2 + \sum b_{kij}X_iX_j \]

\( (k=0, 1, 2, 3, \ldots) \)

Where \( b_{0}, b_{ki}, b_{kii}, b_{kij} \) are constants coefficient and the coded independent variables.

All the independent variables were coded by giving numbers from -1 to +1. The objective of coding the parameters was to weighted value, so that the analysis would not be affected by their individual values. The coded forms of the independent variable were presented in Table 1. The common formula for coding the variable following:
\[ X = \frac{x - (\text{max} + \text{min})/2}{x - (\text{max} + \text{min})/2} \] (3)

Table 1 gives the range of independent variables used in experiments (code and real values). Validation of result was carried out by conducting three representative trials at the optimized conditions. Pectin yield was calculated and residuals were computed to validate the quadratic polynomial model.

Numerical optimization technique of software Design Expert 9.0.4 (Trial version) was used for simultaneous optimization. A suitable combination of pH, solvent: solid ratio, microwave power and time was calculated to have optimum yield value for the pectin yield. All the independent parameters were considered within the range and pectin yield was set for maximization. The desired goals for each response were chosen and different weights were assigned to each factor goal to adjust the shape of its particular desirability. The desirability function approach was applied in the optimization process. This numerical optimization technique evaluates a point that maximizes the desirability function.

2.4 Validation of the Polynomial Model

The polynomial model generated through response surface methodology was validated by conducting 3 trials at the numerically optimized point having maximum desirability.

3. Result and Discussion

3.1 Effect of Processing Parameters on Pectin Yield

3.1.1 Effect of pH on Pectin Yield

It is evident from graph (Fig 1a,b,c, 2a,b,c and 3a,b,c) that as solvent: solid ratio increased from 10 to 30, there was increase in pectin yield from 8.09 to 10.96% at pH 2.5, solvent: solid 20 and extraction time 90 sec. It was observed that power level had significant (p<0.001) effect at 0.1% LOS on pectin yield. It can be concluded that increasing the microwave irradiation energy, the penetration of solvent into the plant matrix can be enhanced and can efficiently deliver to plant cells for pectin extraction. Molecular interaction with the electromagnetic field offers a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted. As a polar solvent, water can efficiently absorb microwave energy and leads to efficient heating. Moreover, the microwave irradiation accelerates cell rupture by sudden temperature rise and internal pressure increase inside the cells of plant sample, which promotes the destruction of sample surface and in turns the exudation of pectin within the plant cells into the surrounding solvents and increase the extraction yield. This phenomenon can explained as following; 540 W was essential for achieving pectin extraction. This corroborates the earlier reports of Maran and Prakash (2015) using a microwave power level of 512 W for optimum pectin yield from waste Carica papaya L. peel.

3.3.4 Effect of Extraction Time on Pectin Yield

The effect of extraction time on pectin yield could be observed from Fig 1a,b,c, 2a,b,c and 3a,b,c. It was observed that as extraction time increased from 30...
to 90 s and the pectin yield increased from 10.28 to 12.83% at pH 1.5, solvent: solid 30, microwave power 720W. Time is one of the important process variables for extraction of pectin and it is associated with the mass transfer effect. The effect of extraction time on pectin extraction was investigated by full factorial ANOVA Table 2. It was observed that time has significant effect on pectin yield for all levels of microwave power, solvent: solid ratio and pH considered. Pectin yield increased rapidly with extraction time. This phenomenon could be explained by the fact that the increase in extraction time increases the reactive site to the effective extraction process. This phenomenon could be explained that, the absorption of microwave energy in the extraction system promoted the thermal accumulation of the extraction solution leading to the dissolution of pectin into the solution. However, the excessive time exposure in the microwave field may cause the degradation of pectin. This result is in agreement with the reports of Maran et al. (2013) who reported optimize time was 169 sec MAE extraction of pectin from orange peel.

### 3.2. Optimization of Pectin Yield

The data was fitted to a polynomial second degree equation. The response was plotted gain the processing variables. Effect of the processing parameters is presented in Fig 4.
The results of analysis of variance (ANOVA) for yield are given in Table 3. The result showed that for above selected range of variables, pH (A), Power (C) and Time (D) had significant (p<0.001) effect at 0.1% LOS, L:S (B) had significant (p<0.019) effect at 1% LOS on pectin yield. Combination of pH and solvent: solid ratio (AB) had significant (p<0.0007) effect at 0.7% LOS, as shown in Table 3. The quadratic term A had highly significant (p<0.0133) effect at 1% LOS and C had highly significant (p<0.0167) effect at...
Fig 4: Effect of processing pH, solvent: solid, microwave power and extraction time on the pectin yield

Non significant effect was observed for all other interactions. It was observed that pH had significant –ve and solvent: solid, microwave power level and extraction time had significant +ve effect on the pectin yield. The Model F-value of 17.42 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, B, C, D, AB, A², C² are significant model terms. Values greater than 0.10 indicate the model terms are not significant. Insignificant model terms were removed and the final form is presented in Table 3. The "Lack of Fit F-value" of 206.22 implies the Lack of Fit is significant.
Table 3: ANOVA for Response Surface Reduced Quadratic model

<table>
<thead>
<tr>
<th>Source</th>
<th>Coefficient Estimate</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value Prob&gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>10.65</td>
<td>144.65</td>
<td>7</td>
<td>20.66</td>
<td>17.42</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>A-pH</td>
<td>-1.01</td>
<td>54.92</td>
<td>1</td>
<td>54.92</td>
<td>46.29</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>B-L:S</td>
<td>0.35</td>
<td>6.81</td>
<td>1</td>
<td>6.81</td>
<td>5.74</td>
<td>0.0190</td>
</tr>
<tr>
<td>C-Power</td>
<td>0.65</td>
<td>22.52</td>
<td>1</td>
<td>22.52</td>
<td>18.98</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D-Time</td>
<td>0.77</td>
<td>32.43</td>
<td>1</td>
<td>32.43</td>
<td>27.34</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AB</td>
<td>0.64</td>
<td>14.85</td>
<td>1</td>
<td>14.85</td>
<td>12.51</td>
<td>0.0007</td>
</tr>
<tr>
<td>A²</td>
<td>0.61</td>
<td>7.62</td>
<td>1</td>
<td>7.62</td>
<td>6.42</td>
<td>0.0133</td>
</tr>
<tr>
<td>C²</td>
<td>-0.59</td>
<td>7.09</td>
<td>1</td>
<td>7.09</td>
<td>5.98</td>
<td>0.0167</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>93.70</td>
<td>79</td>
<td>1.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td></td>
<td>93.70</td>
<td>73</td>
<td>1.28</td>
<td>206.22</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pure Error</td>
<td></td>
<td>0.037</td>
<td>6</td>
<td>0.0062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td></td>
<td>238.38</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Validation of the modified polynomial model for pectin extraction in convectional microwave oven

<table>
<thead>
<tr>
<th>Responses</th>
<th>Observed ± std dev</th>
<th>Predicated</th>
<th>Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin Yield</td>
<td>13.32±0.025</td>
<td>13.47</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Fig 5: Predicted pectin yield Vs Actual pectin yield
There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise. The "Pred R-Squared" of 0.524 is in reasonable agreement with the "Adj R-Squared" of 0.572; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. This ratio of 18.598 indicates an adequate signal. This model can be used to navigate the design space.

The regression equations describing the effect of process variables on pectin yield of pectin extraction in terms of coded levels variable are given as:

\[
\text{Pectin yield} = 10.65 - 1.01(A) + 0.35(B) + 0.65(C) + 0.77(D + 0.64 AB + 0.61 A^2 - 0.59 C^2)
\]

ANOVA for pectin yield was carried out to check the effect of on solvent: solid ratio on pectin yield. It was observed that solvent: solid ratio had significant (p<0.001) effect pectin yield at 0.1% LOS.

Adequate precision value was obtained as >4, demonstrate a significant and intense correlation between the observed and predicted values. The actual and predicted pectin yields are plotted in Fig 5. The data points on this plot lie reasonably close to the straight line and indicate that an adequate agreement between real data and the data obtained from the model.

The optimum extraction conditions and the maximum yield of pectin were obtained from the desirability function approach, having a desirability of 0.717, was pH of 1.5, solvent: solid ratio 20, microwave power of 630 W and extraction time of 89 sec, and the optimize yield of pectin was 13.32 %. The surface graphs generated for different factors were presented in Fig 5.

3.3 Validation of Quadratic Model

The validation of the optimum solution was done by conducting the experiment at above the optimum conditions generated numerically. It had been observed that result obtained were near about similar to optimum solution which is presented in Table 4. The photograph of product obtained at optimized condition is presented in Plate 1.

4. Conclusions

The optimum extraction conditions and the maximum yield of pectin were obtained from the desirability function approach, having a desirability of 0.717, was pH of 1.5, solvent: solid ratio 20, microwave power of 630 W and extraction time of 89 sec, and the optimize yield of pectin was 13.32 %. Thus, it can be hypothesized that by making it a continuous process or industrial scaling up, energy, time and solvent requirement can be lowered to many extents. Pectin powder can be produced microwave based extraction as convenient pectin manufacturing process by the food industry.

Acknowledgements

The authors are grateful to Dr. K. K. Singh, Director, ICAR-CIAE, Dr. Nachiket Kotwalwale, Head, APPD, Dr. Punit Chandra, Dr. Sumedha Deshpande and Dr. Dipika Agrahar-Murugkar for providing laboratory facilities. The authors also wish to acknowledge the help and support received from the Dean, Associate Dean and other staffs of College of Agricultural Engineering and Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbani.

References


Guo X, Han D, Xi H, Rao L, Liao X, Hu X and Wu J (2012). Extraction of pectin from navel orange peel assisted by...


