Pectin from muskmelon (*Cucumis melo* var. *reticulatus*) peels: extraction optimization and physicochemical properties

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Abstract Pectin derived from plant waste sources is currently focused as an economical and eco-friendly approach. Optimization of pectin extraction from muskmelon peel by response surface methodology (RSM) was investigated in this study. Box–Behnken Design (BBD) was used to identify the optimal level of the extraction variables such as time, pH and temperature. A second-order model equation for pectin extraction was obtained from multiple regression analysis of experimental data with the correlation coefficient (R^2) value of 0.92. ANOVA results showed that linear effect of temperature and combined effect of pH with temperature were found significant for pectin extraction from muskmelon peel. Validation results had good agreement with the predicted results. Pectin extracted from muskmelon peel was classed as high methoxy pectin with the equivalent weight of 384.5 g/mol. Non-newtonian pseudoplastic flow behaviour was observed for muskmelon pectin from the viscosity studies.

Keywords Optimization · Extraction · Pectin · Muskmelon · Pseudoplastic · RSM

Introduction

Pectin is a structurally complex heteropolysaccharide present in the peels and skins of the higher plants. Pectin is used in food industry as a thickener, emulsifier, texturizer, stabilizer, gelling agent, confectionery and fat substitute in spreads, ice-cream and salad dressings (Thirugnanasambandham et al. 2014; Minjares-Fuentes et al. 2014). It is also used to treat high blood cholesterol level, hypoglycemia, macrophage induction, heart disease, gallstones and cancer (Zhang et al. 2015; Thirugnanasambandham et al. 2014). Pectin backbone structure is made up of homogalacturonan giving 65% contribution with D-galacturonic acids linked via alpha 1-4 glycosidic linkage. About 20–35% is composed of ramified structure of rhamnogalacturonan-I with varied side chains like galactan, L-Arabinogalactan type I and II based on plant sources and its growing conditions (Zhang et al. 2015).

Various plant sources such as citrus peel, apple pomace (Wikiera et al. 2016), sugar beet, grape peel (Wang et al. 2016), passion fruit peel (de Oliveira et al. 2016; Liew et al. 2014), mango peel (Pandit et al. 2015), banana peels (Oliveira et al. 2015; Qiu et al. 2010) and pumpkin (Minjares-Fuentes et al. 2014) were reported for pectin extraction in the literature. Recent trend is to find gelling agent availability in novel plants and fruit wastes to meet the industrial demands. Muskmelons which fall under Cucurbitaceae family, an annual climber growing to 1.5 m with leaves oval to kidney shaped and grooved fruit with a thick flesh, warty, scaly rind and orange or green flesh grown in summer season, are traded internationally. *Cucumis melo*. L contains 64 g of linoleic acid per 100 g of total fatty acids (Albishi et al. 2013). The fruit contains 3.5% carbohydrates, 0.2% fat, 0.6% protein, 32 mg calcium, 14 mg phosphorus, and 26 mg vitamin per fresh fruits (Salunkhe and Kadam 1998).

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The majority of pectin extraction was carried out by direct boiling, microwave heating and esterification process (Joye and Luzio 2000; Kulkarni and Vijayanand 2010; Kratchanova et al. 2004; Singthong et al. 2004). The possibility of pectin degradation is unavoidable in direct boiling and microwave-based extraction. Extraction of commercial pectin involves a combination of physicochemical process. Till date conventional corrosive mineral acids like sulphuric, nitric, phosphoric, hydrochloric and tartaric acids are being used to hydrolyze protopectin and extraction of commercial pectin from plant tissue which is of environmental concern. Environmental friendly chemical treatments is a better alternative to extract pectin which generates less acidic waste water and does not create health issues. Use of organic acids at high temperatures, source of raw materials, pH and time of extraction could alter the gelling/emulsification properties of pectin. So far, the yield of pectin using organic acid extraction was found maximum of 14.60 and 18.2% reported by Liew et al. (2014) and Seixas et al. (2014), respectively. Similarly, the maximum yield of citrus peel pectin (CPP) and apple pomace pectin (APP) was 21.95 and 16.68%, respectively (Wang et al. 2014). Therefore, in this study, environmental benign and economical citric acid were used for pectin extraction with high yield, gelling strength and less damage to degree of esterification (de Oliveira et al. 2016; Li et al. 2015; Minjares-Fuentes et al. 2014).

Conventional acid-mediated extraction used in industry and influence of the parameters pH, time, temperature and their interactions need importance as they might result in undesired change of physicochemical properties (Denman and Morris 2015). Statistical experimental methods reduce errors among variable effects (de Oliveira et al. 2016). Optimizing one variable at a time takes large number of experiments to be conducted and seems to be time consuming, which fails to reveal the interaction among variables. These limitations can be overcome by employing response surface methodology (RSM), one of the statistical methods that helps to understand the interaction effects among variables with less number of experimental runs (Srikanth et al. 2014; Padmanaban et al. 2015; Sharmila et al. 2016). Box–Behnken experimental design was performed for three variables such as pH, temperature and time to obtain best possible pectin extraction yield. This approach will help to quantify the parameter values and their interactions. The optimized parameter values will help scientific community for further studies.

The aim of the present study is to optimize the conditions to extract maximum pectin from muskmelon peel. Characterization of the extracted pectin was studied by determining the equivalent weight and methoxy content. Flow behaviour pattern of the muskmelon pectin solution was investigated by viscosity measurements.

Materials and methods

Chemicals

Anhydrous citric acid, 99% ethanol, concentrated sulphuric acid, monohydrate galacturonic acid, carbazole, pectin, sodium hydroxide, sodium chloride and phenol red indicator were purchased from HiMedia (India) and it is of analytical grade.

Collection and processing of muskmelon peel

Muskmelon peels belongs to *Cucumis melo* var. *reticulatus* variety were collected from nearby fruit shop. Then, the peels were cut into very small pieces and subjected to drying under natural sunlight until the moisture content was completely removed. The dried peels were then grinded into powder.

Pectin extraction

5 g of peel powder was accurately weighed and 150 ml of distilled water was added. Anhydrous citric acid powder was added to bring the pH of the solution to 1.5. Mixture was heated at 80 °C for 1 h and it was stirred occasionally. Then, the sample was filtered using muslin cloth and the filtrate was added with equal volume of ethanol and incubated for 3 h at 4 °C to allow the pectin to precipitate. The precipitate was then collected and repeatedly washed with ethanol. After the washing step, the precipitate was dried. Pectin yield was calculated using the following formula (Hosseini et al. 2016a):

$$\text{Pectin yield (\%)} = \frac{\text{Amount of pectin extracted (g)}}{5\text{g of muskmelon peel powder}} \times 100.$$

Optimization by response surface methodology

Box–Behnken design (BBD) was used to study the effects of various parameters on the extraction of pectin using citric acid. In this experimental design, three variables were considered at three levels. All variable values were taken at low, middle and high level. Totally, 17 runs were done as a part of experimental design. Yield of pectin from each run was determined as response and the surface plots were used to study the effect of interactions of two variables.

Assays

Galacturonic acid content determination

10 mg of dried pectin sample was weighed and dissolved with 10 ml of 0.05 N NaOH. The solution was allowed to stand for 30 min for deesterification of pectin. 0.2 ml of

this solution was taken and added with 9.8 ml of distilled water. 2 ml of this solution was taken and 1 ml of carbazole reagent was added to it and the formation of white precipitate was observed. Then, 12 ml of concentrated sulphuric acid was added under constant stirring. The solution was allowed to stand for 10 min to develop colour. The colorimetric reading was taken at 525 nm. In blank, 1 ml of 99% ethyl alcohol was added in the place of carbazole reagent. The concentration of galacturonic acid was determined using the calibration graph made with galacturonic acid (12–96 µg/ml) standard solutions (Sadasivam and Manickam 2008).

Equivalent weight determination

5 ml of ethanol was added to accurately weighed 0.5 g of pectin sample. The sample was added with 1 g of NaCl and dissolved with 100 ml of distilled water. The solution was titrated against 0.1 N NaOH. Appearance of pink colour was the end point. The end point was indicated using Phenol Red and the equivalent weight was determined using the formula (Sharma et al. 2014):

$$\text{Equivalent weight} = \frac{\text{Weight of pectin (g)}}{\text{Vol of NaOH} \times \text{N of NaOH} \times 1000}.$$

Methoxyl content determination

The neutral solution obtained from equivalent weight determination step was added with 25 ml of 0.25 N NaOH. The solution was mixed thoroughly and allowed to stand for 30 min. 25 ml of HCl was added before titrating against 0.1 N NaOH. Phenol Red was used as the indicator. The methoxyl content was calculated using the formula (Castillo-Israel et al. 2015; Owens et al. 1952):

$$\text{Methoxy content (\%)} = \frac{\text{Vol of NaOH} \times \text{N of NaOH} \times 3.1}{\text{Weight of pectin (g)}}.$$

Characterization

Fourier transform infrared spectroscopy (FTIR) was used to characterize the pectin extracted from muskmelon peel. FTIR analysis was performed in Spectrum Two model (Perkin Elmer) by Easy diffusion procedure. The pectin samples and KBr were mixed in 1:99 ratio and the mixture was kept in the diffusion sample holder to obtain the FTIR spectra with the scan range of 450–4000 cm⁻¹.

Viscosity studies

0.5 g of pectin was dissolved in 100 ml of 0.9% NaCl solution and the viscosity was determined using Redwood

viscometer. The viscosity was measured at different temperature from 40 to 80 °C in the intervals of 10 °C and the corresponding time taken for the collection of 50 ml of sample was noted. The graph between the apparent viscosity and shear rate was plotted to determine the fluid type of pectin solution.

Results and discussion

Optimization of pectin extraction by RSM

The process variables such as time, pH and temperature were optimized by employing RSM for pectin extraction from muskmelon peels. The RSM optimization studies using BBD were done with proposed range of the significant factors such as time, pH and temperature were 30–60 min, 1–2 pH and 70–90 °C, respectively. The RSM distribute the process variables as following low (−1), mid (0) and high levels (+1) for (A) Time: 30 min (−1), 45 min (0) and 60 min (+1); (B) pH: 1 (−1), 1.5 (0) and 2 (+1); (C) Temperature: 70° C (−1), 80° C (0) and 90° C (+1), respectively. The software minimizes the number of experiments for the input data and generates a table consisting of 17 experimental runs which include five replicates around the centre point (0, 0, 0) as shown in Table 1. Based on the suggested data table from software, the experiments were conducted and the pectin extraction results were analysed to develop an ANOVA statistics (Table 2) and a second-order polynomial model equation (Eq. 1)

$$\begin{aligned} \text{Pectin yield (\%)} = & 2.44 - 0.14A + 0.025B - 0.29C \\ & - 0.10AB + 0.075AC + 0.60BC \\ & + 0.34A^2 + 0.62B^2 - 0.11C^2. \end{aligned} \quad (1)$$

The peak pectin extraction (3.8%) was achieved with the runs 3 and 9 (Table 1). The results of the experimental data and predicted data were juxtaposed and ensured the significance of the experiments done. A view of the data obtained put together gives a clear idea about the role of an independent variable in the enhancement of the pectin extraction. The obtained results showed that the time and pH do not affect the improvement of pectin yield. In contrast, the higher productivity was observed at a low level and a mid-level range of temperature but not at high level indicating that temperature appears to have a marginal effect.

The quadratic model results generated by the Design-Expert 7 software were analysed using ANOVA (Table 2). The individual effect, the effect of interaction coefficients

Table 1 BBD matrix for pectin extraction from muskmelon peel

Run order	A (time) (min)	B (pH)	C (temperature) (°C)	Pectin yield (%)	
				Experimental	Predicted
1	30	1	80	3.4	3.4
2	60	1	80	3.2	3.3
3	30	2	80	3.8	3.7
4	60	2	80	3.2	3.2
5	30	1.5	70	3.2	3.2
6	60	1.5	70	2.9	2.8
7	30	1.5	90	2.3	2.5
8	60	1.5	90	2.3	2.3
9	45	1	70	3.8	3.8
10	45	2	70	2.5	2.7
11	45	1	90	2.2	2.0
12	45	2	90	3.3	3.3
13	45	1.5	80	2.4	2.4
14	45	1.5	80	2.4	2.4
15	45	1.5	80	2.8	2.4
16	45	1.5	80	2.5	2.4
17	45	1.5	80	2.1	2.4

Table 2 ANOVA statistics for pectin extraction from muskmelon peel

Source	Sum of squares	Df	Mean square	F value	p value Prob > F	Significance
Model	4.53	9	0.50	9.05	0.0042	Significant
A (time)	0.15	1	0.15	2.72	0.1432	
B (pH)	0.005	1	0.005	0.09	0.7731	
C (temperature)	0.66	1	0.66	11.88	0.0107	Significant
AB	0.04	1	0.04	0.72	0.4246	
AC	0.022	1	0.022	0.40	0.5451	
BC	1.44	1	1.44	25.88	0.0014	Significant
A ²	0.49	1	0.49	8.88	0.0205	Significant
B ²	1.61	1	1.61	28.85	0.0010	Significant
C ²	0.049	1	0.049	0.87	0.3808	
Residual	0.39	7	0.056			
Lack of fit	0.14	3	0.046	0.73	0.5867	Not significant
Pure error	0.25	4	0.063			
Total	4.92	16				

 $R^2 = 0.92$

and overall quadratic model for the process variables such as Time (*A*), pH (*B*) and Temperature (*C*) were proposed through *F* and *p* values as output. The *p* value, $p < 0.05$, $0.05 < p < 0.1$ and $p > 0.1$ indicates that the coefficient terms are significant, slightly significant and insignificant, respectively. Following the conditions of *p* value (Prob > *F*), ANOVA was deduced to found that *C* (temperature) and *BC* (pH and temperature) are significant

model terms, whereas *A* (time), *B* (pH), *AB* (time and pH) and *AC* (time and temperature) were found insignificant. High *F* value (90.49) and low *p* value (0.004) suggested that the obtained regression model was statistically significant. The Adequate precision is 9.811 (signal to noise ratio >4 is considered as desirable while measuring the Adeq. precision) and coefficient of variation is low, which was found to be 8.30% indicating that the model is more

than the adequate adjustment of the quadratic model to the obtained experimental data. The precision of the model over the range of data obtained was analysed by the predicted vs actual plot. The plot exhibits the scatter points of data about the diagonal line and the cluster of points over or under the line indicates the difficulty of above or below prediction. Figure 1a shows that the line passing through the entire range of data points suggests that the model is good fit. The comparative effects of the process parameters involved in the production of pectin were analysed using perturbation plot and suggested that the change in response of each factor with other corresponding factors fixed constant from the chosen reference point. The steep curvature indicates that the production of pectin is very sensitive to the pH, moderate sensitive to time and less sensitive to temperature (Fig. 1b). The 3D response surface plots are used to investigate the interactions amongst the process variables and to determine the optimal conditions for the maximum pectin yield (Fig. 2a–c). In Fig. 2a–c, when two variables are in interaction, the third variable was kept constant at middle level as hold values, i.e. (time = 45 min; pH = 1.5; temperature = 80 °C).

Effect of time and pH

The 3D plot *AB* depicts the interaction between *AB* (Time vs pH), indicating that a high pH level (2) and time (60 min) showed an increase in pectin production (Fig. 2a). The effect of interaction between time (*A*) and pH (*B*) yielded a less significant result. The maximum yield (3.45%) was observed as pH and time were increased. Lower pH was favoured for extraction because of hydration of carboxyl acidic groups of pectin by the excess hydrogen ions of solvent. As the carboxyl group lost their charges they tend to reduce the repulsive forces which facilitated the precipitation of pectin easily. Thus, lowering the pH

and prolonging the time of extraction yielded more pectin (3.31%). However, after certain period saturation may occur. Optimum extraction time was found to be 60 min in this study. Pectin from papaya peel was studied by Liew et al. (2014) and found that optimum condition for extraction was pH 2.0 and extraction time 60 min. Similar studies reported as an increase in pH with time improve the yield of pectin (Maran 2015; Oliveira et al. 2015).

Effect of time and temperature

The interaction effect of two variables time (*A*) and temperature (*C*) had less significance on experimental process (Fig. 2b). Maximum yield (3.15%) was obtained at low temperature (70 °C) and less time of exposure (30 min). The yield increases with increasing time and decreasing temperature. Long extraction period with high temperature would have a thermal degradation effect on pectin so that it cannot be precipitated by alcohol and the pectin yield will be reduced. Similar result of extraction time on pectin extraction from cacao pod husks was reported by Vriesmann et al. (2012).

Effect of pH and temperature

The 3D plots representing a significant interaction coefficient between *BC* (pH vs temperature) suggest that the productivity of pectin increases with increasing the levels of two variables temperature and pH with fixed level of time (Fig. 2c). The interaction effect of two variables pH (*B*) and temperature (*C*) had more effect on the pectin extraction. Maximum yield (3.76%) was obtained when the temperature and pH were at low level. The yield decreases with increase in temperature and increase in the temperature had degrading effect on the pectin. Low pH and temperature facilitate the extraction of pectin. Tang et al.

Fig. 1 **a** Actual versus predicted response plot and **b** perturbation plot for pectin extraction from muskmelon peel

Fig. 2 3D surface plots showing the interaction effects of **a** time and pH, **b** time and temperature and **c** pH and temperature on pectin extraction from muskmelon peel. The third variable in each plot was

(2011) studied the extraction of pectin from dragon fruit peel and found that the maximum yield was obtained at pH 1.5 and temperature 80 °C.

Model validation

The numerical optimization tool of Design Expert 7 software was used to identify the optimal level of time, pH and temperature for maximized output. The ramp plot for optimal conditions is shown in Fig. 3, and showed that the maximum pectin yield (3.983%) was predicted at time (60 min), pH (1.01) and temperature (70.53 °C). To verify the model, the validation experiments were performed at predicted optimal levels of selected variables and 3.24% of pectin yield was obtained. The obtained model was fitted well the experimental results with 81.3%. At optimal conditions, the galacturonic acid content of the extracted pectin was determined to be 47%. This optimization study for the extraction of pectin from musk melon was correlated with the reports published previously (Liew et al. 2015; Li et al. 2015; Casas-Orozco et al. 2015; Korish 2015a, b). The method of extraction is placed in the core for the better yield and quality of pectin. Direct boiling and microwave and ultrasound-assisted extraction are commonly used techniques according to the literature (Minjares-Fuentesa et al. 2014; Yeoh et al. 2008; Joye and Luzio 2000). Though better yield was obtained in these methods, each method has some limitations. In direct boiling, there is a chance of thermal degradation of pectin due to exposure for more than 2 h. Similar effects were observed with microwave and ultrasound-assisted extraction since the rise in temperature during the process degrades the pectin. In this study, the citric acid-based extraction was found to be better method for pectin extraction from muskmelon peels.

kept constant at middle level as hold values i.e. (time = 45 min; pH = 1.5; temperature = 80 °C)

Fig. 3 Ramp desirability plot for numerical optimization representing the optimal level of selected variables (time, pH and temperature) for maximized pectin extraction

Characterization

The pectin extracted from muskmelon was characterized by FTIR analysis. The peak positions of pure pectin and the extracted pectin from muskmelon peel showed high similarity (Fig. 4). The broad band appeared at 3429 cm^{-1} in the spectra corresponds to the OH groups due to hydrogen intra- and intermolecular hydrogen bonding of pectin (Seslija et al. 2016). The presence of alkyl group was observed by the existence of peak at 2921 cm^{-1} . The functional identity of pectin was confirmed by the observation of peaks for esterified carboxyl groups (1740 cm^{-1}) and free carboxyl groups (1634 and 1145 cm^{-1}) in the FTIR spectrum. The absorption peak at 1331 is assigned to the stretching COO-functional group of pectin. Since the functional groups of the pure pectin were present in the extracted pectin sample, FTIR analysis confirmed that the extracted polysaccharide from muskmelon peel was pectin without any impurities.

Fig. 4 FTIR spectra of
a commercial pure pectin and
b muskmelon peel pectin

Physicochemical properties of pectin

Equivalent weight

The equivalent weight was the total content of galacturonic acid. The equivalent weight value depends on the pH and extraction solvent used. The value of equivalent weight was also found to be dependent upon amount of free acids available. The lower value of equivalent weight was due to the polymerization of pectin at lower pH. The equivalent weight value of pectin extracted from muskmelon peel was calculated using the formula and value was found to be 384.5 g/mol. The obtained equivalent weight of pectin in this study was in accordance with the results reported on pectin extraction from papaya and guava peel using citric acid (Liew et al. 2014).

Methoxyl content

The neutral solution from equivalent weight determination was taken for methoxy content calculation. Methoxy content was calculated by titrimetric method and the value was calculated as 61.38%. Methoxyl content of pectin determines the gel strength, setting time and sensitivity towards metal ions. The value of methoxyl content varies with source and extraction conditions. Based on the degree of methylation (DM), pectins are classified into high-methoxyl (HM) pectins and low-methoxyl (LM) pectins. When degree of methylation of pectin is 60–70% or more, they are classified as high-methoxyl pectins which can form higher sugar gels with rapid setting time of 20–70 s. Pectins lesser than 50% degree of methylation fall under low-

methoxyl pectins also describes low concentration sugar gels having slow set time of 180–250 s. Alba et al. (2015) reported low methoxy pectin (LMP) extracted from okra pods with low methoxyl content (40%) and high methoxy pectin (HMP) from passion fruit peel was reported by Liew et al. (2014) with value nearly 54.78%. In this study, the pectin extracted from muskmelon peel was found to be high methoxy pectin (HMP) with methoxyl content of 61.38%.

Viscosity studies

The viscosity was determined using redwood viscometer at different temperature to study the flow behaviour of 0.5% pectin solution. The results explained that the shear rate was decreased as the temperature was increased. Figure 5 represents the relation between the viscosity and the shear rate and revealed that the viscosity was decreased with increase in shear rate. The non-newtonian fluid parameters like consistency index (K) and flow behaviour index (n) were determined by employing power law model. The apparent viscosity of power law fluids can be expressed as (Doran 2013):

$$\mu = \frac{\tau}{\gamma} = K\gamma^{n-1} \quad (2)$$

where μ is the viscosity, τ is the shear stress, γ is the shear rate, K is the consistency index and n is the flow behaviour index. Equation 2 was linearized by taking 'ln' on both sides to get Eq. 3

$$\ln \mu = \ln K + (n - 1) \ln \gamma. \quad (3)$$

Fig. 5 Plot representing the relation between **a** viscosity and shear rate and **b** $\ln \mu$ vs $\ln \gamma$

The values of K and n can be determined from the intercept and slope of the graph plotted between $\ln \mu$ vs $\ln \gamma$. From Fig. 5, the consistency index (K) and flow behaviour index (n) were determined as 0.042 and 0.689, respectively. Since $n < 1$, the flow pattern of pectin obtained from muskmelon peel was found to be non-newtonian pseudoplastic type. Previous viscosity studies on plant derived pectin were reported as pseudoplastic flow behaviour (Hosseini et al. 2016a, b; Chen et al. 2014).

Conclusion

A simple and safer extraction method was developed to extract pectin from muskmelon peel. The process conditions (time, pH and temperature) were optimized by BBD of RSM and the results were analysed. ANOVA results revealed that the linear effect of temperature and interaction effect of pH and temperature were significant. The predicted and experimental results have good correlation

with R^2 value of 0.92. The pectin was characterized as high methoxy pectin with the equivalent weight of 384.5 g/mol. Results of this study revealed that the muskmelon peel can be utilized as cheap source for pectin extraction using citric acid and the obtained pectin can be used in food and biopolymer industries.

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Compliance with ethical standards

Conflict of interest The authors declared that there is no conflict of interest on publication of this article.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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