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Microwave optimization of mucilage extraction from *Opuntia ficus indica* Cladodes

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ABSTRACT

In this study, microwave-assisted extraction (MAE) of polysaccharides from *Opuntia ficus indica* Cladodes were investigated using response surface methodology (RSM). The effects of three extraction factors on the yield of mucilage were examined. The results indicated that the optimum extraction conditions were determined as follows: microwave power X_1, 700 W; extraction time X_2, 5.15 minand ratio water/raw material X_3, 4.83 mL/g at fixed pH 11. Under these optimal extraction conditions, mucilage yield was found to be Y, 25.6%. A comparison between the model results and experimental data gave a high correlation coefficient (R^2 = 0.88), adjusted coefficient (R_{adj} = 0.83) and low root mean square error (RMSE = 2.45) and showed that the two models were able to predict a mucilage yield by green extraction microwave process.

1. Introduction

Prickly pads are grown throughout Mexico and in all North and South American. This cactus grows in many other regions of the World such as Africa, Australia and in the Mediterranean area [1,2]. This crop has been used in some countries as vegetable or for the consumption of its natural fruits. Cactaceae family is characterized by mucilage production, this compound is secreted by mucilaginous cells located in the chlorenchyma and parenchyma tissues [3]. Mucilage biosynthesis takes place in specialized cells which excrete it into the apoplast [4], its physiological role has been associated with their ability to bind water under unfavorable climatic conditions [5].

Mucilage is a polysaccharide containing a molecular structure up to 30,000 monosaccharides [6]. This polymer has a highly branched structure that contains residues of arabinose, galactose, galacturonic acid, rhamnose and xylose [7,8]. According to Felkai-Haddache, Remini, Dulong, Mamou-Belhabib, Picton, Madani and Rihouey [9], the polymers composing the mucilage are of high molecular weight polymers with molecular weight ranged from 15.3 to 15.7×10^6 g mol^{-1}, the rest are polymers with low molecular weight polymers that acts as a polyelectrolyte.

This carbohydrate is considered a potential source of industrial hydrocolloids [3]. Multiples uses have been found for this component for instance as a food thickener, food emulsifier, as a water purifier, as an adhesive for lime, as a natural super-plasticizer in mortar and as food product [10,11].

The method commonly used for extraction of the mucilage from *Opuntia ficus indica* (*OFI*) cladode consists in maceration of the mixed cladode in distilled water at room temperature or under heating [10–13], however, this method is effective, long and may prove to be costly. Microwave assisted extraction (MAE) is an alternative for extraction of substance from materials, which has characteristics of the economization of the extracting time, solvent amount and energy consumption [14,15].

In the previous work about MAE impact on the physico-chemical and rheological quality of mucilage extracted from the *Opuntia ficus indica* cladode, Felkai-Haddache, Remini, Dulong, Mamou-Belhabib, Picton, Madani and Rihouey [9] have shown that MAE is an appropriate method to extract mucilage. MAE extracts more

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efficiently polysaccharides molecules and should be preferred based on the lower extraction time and extractability quality [9]. The MAE performance depends on its operational mode of heating, and provides pulsed microwave heating at certain power which is efficient in extracting thermal-labile compounds [16]. The increasing microwave irradiation power enhance the penetration of solvent into the plant matrix, which allow the dissolution of components to be extracted [17]. After obtaining higher extraction yield related to the time-power exposure; previous works have shown that the excessive time-power exposure under the microwave fields, leads to degrade the polysaccharide molecules [14].

The aim of the present work is the optimization of microwave assisted extraction of mucilage 'MAE' from *Opuntia ficus indica* peeled cladodes, using response surface methodology (RSM), to study the effects of extraction power, extraction time, pH and water to the raw material ratio on the mucilage recovery.

2. Materials and methods

2.1. Materials

Opuntia ficus indica cladodes were harvested from Aokas (Bejaia) located in North-East of Algeria during the period of October 2014. The cladodes were studied without thorns and epidermis; in this case, approximately 35% of the epidermis in both sides was removed. The average physical dimensions of the selected cladodes were 345 ± 3 mm-long, 160 ± 5 mm-wide and 18 ± 4 mm-thick; the initial mass was 930 ± 12 g for the intact cladodes, and 605 ± 10 g in those cases when the epidermis were removed. Cladodes were rinsed with distilled water (0.5 μS/cm), disinfected with ethanol 70% (v/v) and the epidermis were removed. The peeled cladodes were then mixed in a domestic mill (Moulinex MASTERCHEF 370, Alençon, France). The obtained homogenates were stocked at 4 °C for further uses.

2.2. Microwave assisted extraction of mucilage

Microwave assisted extraction (MAE) from cladodes were performed in domestic microwave oven system (2.45 GHz, Samsung Model NN-S674MF, Kuala Lumpur, Malaysia). The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter was linearly adjustable from 100 to 1000 W). The oven was modified (Fig. 1) in order to condense the vapors generated during extraction into the sample. The MAE procedure was performed in a 500 mL volumetric flask.

2.3. Mucilage precipitation

The extracted mucilage was immediately cooled in ice bath (4 °C), filtered through a double layer cheeseclothto remove pulp and centrifuged at 4000 × g during 15 min at 4 °C (Sigma 2-16 PK model, Osterode am Harz, Germany). The filtrate was precipitated in three volumes of ethanol 95% (v/v) at 4 °C overnight. The precipitate was washed three times with ethanol (75%, v/v) then desolvated by lyophilization at −55 °C for 12 h (MartinChrist, Freeze Dryer Alpha 1–2/LD Gefriertrockungsanlagen GmbH, Osterode am Harz, Germany) as reported by Matsuhiro, Lillo, Sáenz, Urzúa and Zárate [18].

2.4. Calculation of extraction mucilage yield

Extraction mucilage yield was the ratio of the dry mass lyophilized extract (DMLE), on the dry weight of the dehydrated peeled cladodes (DWDPC) dried at 73 ± 1 °C in a vacuum oven as outlined by Sepúlveda, Sáenz, Aliaga and Aceituno [13].

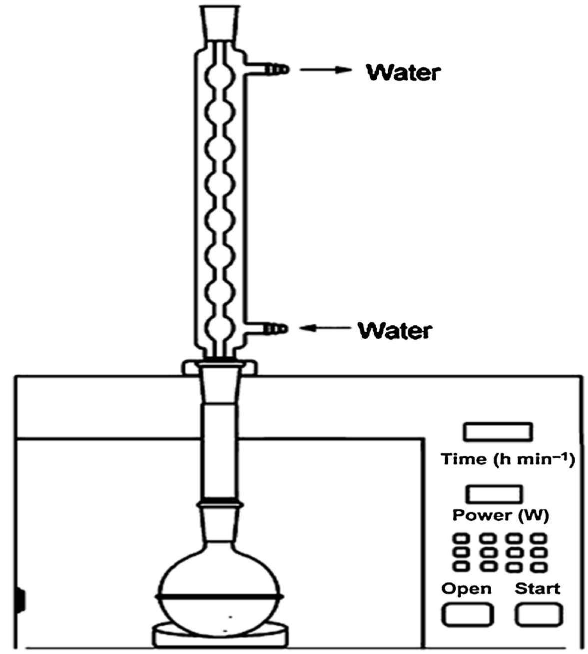

Fig. 1. Sketch of a modified microwave apparatus [30].

2.5. Experimental optimization

2.5.1. Preliminary trials

Before optimization of the microwave assisted extraction of mucilage from *OFI* peeled cladode, the influences of the process parameters were initially separately investigated in single-factor experiments to limit the total experimental work. When one variable was not studied, it was kept constant. Microwave power, irradiation time, pH and mixed cladode–water ratio are selected as independent variables, whereas mucilage extracted yield (EYM) is selected as response.

To investigate the effect of microwave power and irradiation time on mucilage yield, pH and mixed cladode–water ratio were set at 5 and 1:3 (v/v), respectively. To investigate the effect of mixed cladode–water ratio, the microwave power, irradiation time and pH were fixed at 900 W, 3 min and 5, respectively. To investigate the effect of pH, the microwave power, irradiation time and mixed cladode–water ratio were fixed to 900 W, 3 min and 1/4 (v/v), respectively.

2.5.2. Experiment design (RSM optimization)

On the basis of the single-factor experimental results, the factor levels corresponding to each independent variable (Microwave power, irradiation time and mixed cladode–water ratio) were chosen. The value of pH is fixed at 11 for all experiments (Table 1).

A response surface methodology based on a Box–Behnken Design (BBD) for MAE of mucilage yield was conducted to optimize the process (Table 1). The factor levels were coded as −1 (low), 0 (central point or middle) and 1 (high), respectively. Maximum information using minimum trials is obtained by experimental design based on mathematical rules. The RSM is a set of mathematical techniques in which a response of interest is influenced by several variables [19]. It can help in investigating the interactive effect of process variables and in building a mathematical model that accurately describes the overall process, allowing process optimization to be conducted effectively [20].

Regression analysis of the data to fit a second-order polynomial equation (quadratic model) was carried out according to the

Table 1
The experimental design layout and corresponding responses for Box-Behnken design (BBD) based on response surface methodology (RSM) for Mucilage recovery compounds *Opuntia ficus indica* (*OFI*) cladodes samples using microwave assisted extraction (MAE) (in duplicate).

X_1-Extraction time (min)	X_2-Microwave power (W)	X_3-Ratio water/ material (mL/g)	Y-Mucilage recovery (%)
1	500	3	12.29 ± 1.218
1	700	5	15.71 ± 0.399
7	500	3	18.65 ± 0.144
4	700	3	23.64 ± 0.043
1	500	3	12.29 ± 0.057
4	900	5	22.91 ± 0.646
4	500	1	10.38 ± 1.184
4	700	3	22.64 ± 1.298
4	700	3	23 ± 1.016
1	900	3	13.01 ± 0.750
1	900	3	13.09 ± 0.241
4	700	3	17.45 ± 1.259
7	700	1	10.7 ± 0.293
7	700	5	25.56 ± 0.209
1	700	1	8.48 ± 1.673
4	500	1	9.46 ± 0.434
7	700	1	10.13 ± 0.598
4	900	1	9.89 ± 1.111
4	700	3	23.05 ± 0.521
7	700	5	22.58 ± 1.211
7	900	3	6.82 ± 0.065
7	900	3	9.19 ± 0.131
1	700	5	13.79 ± 1.043
4	500	5	18.62 ± 0.654
4	900	1	10.09 ± 1.002
4	900	5	21.23 ± 0.821
4	500	5	16.9 ± 1.213
4	700	3	23.64 ± 1.076
1	700	1	8.18 ± 0.054
7	500	3	20.08 ± 1.076

following general equation (Eq. (1)), which was then used to predict the optimum conditions of MAE of mucilage recovery.

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X^2 + \sum_{i>j}^{k} B_{ij} X_i X_j + E \quad (1)$$

Where Y represents the response function (in our case turbidity removal); B_0 is a constant coefficient; B_i, B_{ii} and B_{ij} are the coefficients of the linear, quadratic and interactive terms, respectively, and X_i and X_j represent the coded independent variables. According to the analysis of variance, the regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions, the regression coefficients were used to generate 3-D surface plots from the fitted polynomial equation. The variables were coded according to the following equation (Eq. (2)):

$$X_i = \frac{x_i - x_0}{\Delta x} \quad (2)$$

where X_i is the (dimensionless) coded value of the variable x_i, x_0 is the value of x at the center point and Δx is the step change. Analysis of variance was performed for the response variable using the full model where p-value (partitioned into linear and interaction factors) indicated whether the terms were significant or not. To verify the adequacy of the models, MAE treatment trials were carried out at the optimal conditions predicted by the RSM and the obtained experimental data were compared to the values predicted by the regression model.

Efficiency of MAE treatment was evaluated based on the mucilage recovery measured under the optimum conditions selected by RSM.

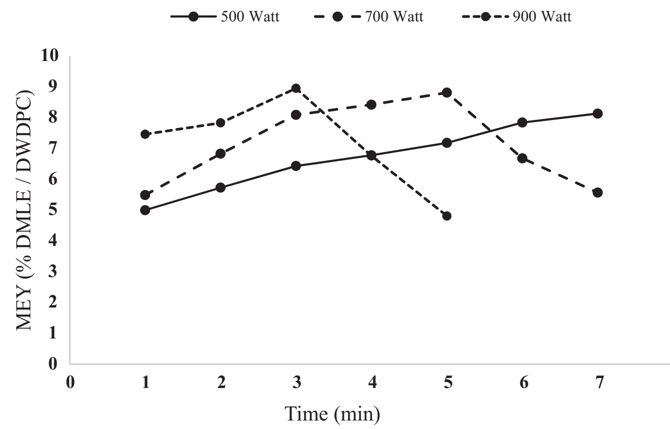

Fig. 2. Microwave power and time irradiation impact on the mucilage extracted yield. DMLE: Dry Mass Lyophilized Extract, DWDPC: Dry Weight of the Dehydrated Peeled Cladodes.

2.6. Statistical analysis

Data obtained from the BBD was statistically analyzed using ANOVA for the variable in order to test the model significance and suitability. All extraction process and yield results were expressed as the means of two replications. $p < 0.05$ and $p < 0.01$ were taken as significant and highly significant levels, respectively. The JMP (Trial Version 10, SAS, USA) software was used to construct the BBD and to analyze all the results.

3. Results and discussion

3.1. Microwave power and time irradiation effect

The extracted mucilage recovery is increased by increasing the microwave power level as shown in Fig. 2. According to Thirugnanasambandham, Sivakumar and Maran [21], an increase in microwave power will improve the solubility of sample for the enhanced extraction efficiency. The increasing microwave irradiation energy can enhance the penetration of solvent into the plant matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted [17]. As well as increase in microwave power would increase the dipole rotations, which result in power degenerate inside the reaction mixture. This quickly generates a heat in reaction mixture, thus yield of polysaccharide is enhanced [22].

To examine the effect of time irradiation on the extracted mucilage yield, experiments were carried out in various extraction time (1–7 min), the results were shown in Fig. 2. The yield of polysaccharide was increased rapidly with increasing extraction time for all powers. It rise from 5 to 8.13%, as time of extraction increased from 1 to 7 min for MAE 500 W, from 5.49 to 8.81% as extraction time increased from 1 to 5 min for MAE 700 W and from 7.46 to 8.95% when the extraction time increase from 1 to 3 min for MAE 900 W. This phenomenon could be explain by the fact that the increase in extraction time increases the reactive site to the effective extraction process, which enhance the yield of polysaccharides [21].

Beyond 3, 5 and 7 min for the powers 900, 700 and 500 W, respectively, the yield started to decrease. According to Zheng, Yin, Liu and Xu [14], the excessive time exposure in the microwave field may cause the degradation of polysaccharides. In previous work, Felkai-Haddache, Remini, Dulong, Mamou-Belhabib, Picton, Madani and Rihouey [9] have shown that MAE gave the highest

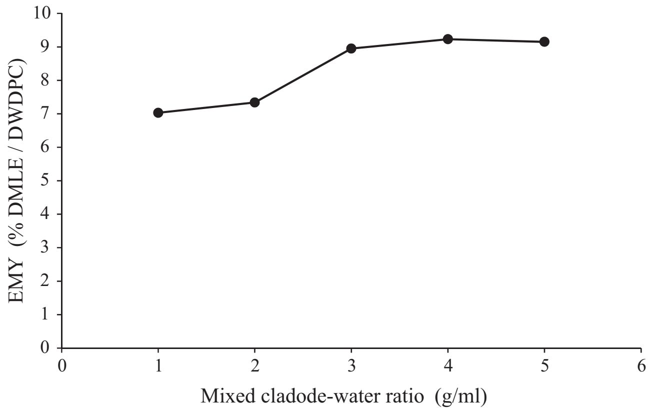

Fig. 3. Water/raw material ratio impact on the mucilage extracted yield. DMLE: Dry Mass Lyophilized Extract, DWDPC: Dry Weight of the Dehydrated Peeled Cladodes.

yield extraction within the lowest extraction time, provided more protein and carbohydrates compared to conventional extractions. Moreover, the obtained polysaccharides from MAE have high molecular weight than mucilage obtained from conventional extraction.

3.2. Water/raw material ratio effect

Different ratio of water to raw material will significantly affect extract yield. If ratio of water to raw material is too small, polysaccharides in raw material cannot be completely extracted up. If ratio of water to raw material is too big, this will cause high process cost. Therefore, suitable ratio of water to raw material should be selected for extraction of targeted polysaccharides [23,24]. The extraction yield of mucilage significantly increases from 7.03% to 9.23% as the ratio of water to raw material increased from 1 to 4 (Fig. 3). This is due to the fact that the increasing of the ratio of water may increase diffusivity of the solvent into cells and enhance the desorption of the polysaccharides from the cells [25]. Bendahou, Dufresne, Kaddami and Habibi [26] reported that the extraction yield of polysaccharides significantly increases as the ratio of water to raw material was increased, which could be due to an increased driving force for the mass transfer of the polysaccharides. However, when the ratio continued to increase, the extraction yields no longer changed.

3.3. pH effect

The extracted mucilage yield, increase proportionally with the increase in pH and then decrease (Fig. 4). The maximum extracted mucilage yield is obtained at pH 11. The increasing of pH leads to the dissociation of acidic groups in polysaccharides and the repulsion between the negative-charged. This also increased the polysaccharides solubility in water and thus allowed to a best yield extraction [27]. At pH > 11, the mucilage yield started to decrease, according to Yang, Wang, Li and Yu [28], the high pH affect the solubility of polysaccharides by decreasing their solubility in the alkaline solution.

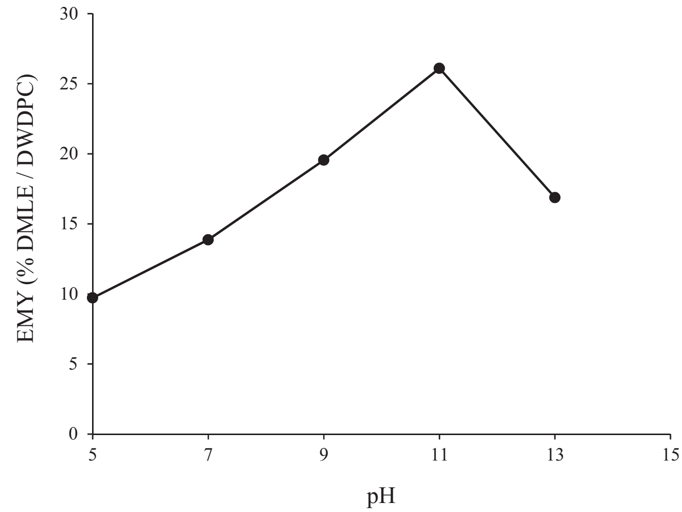

Fig. 4. pH impact on the mucilage extracted yield. DMLE: Dry Mass Lyophilized Extract, DWDPC: Dry Weight of the Dehydrated Peeled Cladodes.

3.4. Model establishment

The BBD matrix and the corresponding results of RSM experiments were shown in Table 1. Through multiple regression analysis on the experimental data (Table 2), the model for the predicted response could be expressed by the quadratic polynomial equations (in the form of coded values) and were achieved as follow:

$$y_{\text{Mucilage yield}\,(\%)} = 22.24 + 1.68X_1 - 0.78X_2 + 4.99X_3 - 3.03X_1X_2 + 1.80X_1X_3 + 1.06X_2X_3 - 4.80X_1^2 - 4.26X_2^2 - 3.04X_3^2$$

In order to determine whether the quadratic model is significant, it is necessary to run ANOVA analysis (Table 3). The results of the second order response surface model fitting in the form of ANOVA are given in Table 3. The ANOVA demonstrated that the model is highly significant with a $p < 0.001$. The determination coefficient ($R^2 = 0.88$) indicates that only 12% of the total variations is not explained by the model. For a good statistical model, the adjusted determination coefficient R^2 adj should be close to R^2. In our model it was 0.83 and then quite close to R^2. Moreover, a root mean square error (RMSE) is in reasonable closed to zero (2.45)

Table 2
Parameters estimate for the experimental results obtained by mathematical model.

Source	Parameters estimate	Standard error	*t*-Ratio	Prob > |*t*|	Confidence interval	
					Lower 95%	Upper 95%
Intercept (B0)	22.24	0.99	22.24	<.0001	20.15	24.32
X_1-Extraction time (min)	1.68	0.61	2.74	0.0126	0.40	2.96
X_2-Microwave power (W)	−0.78	0.61	−1.27	0.2188	−2.05	0.49
X_3-Ratio water/material (mL/g)	4.99	0.61	8.16	<.0001	3.72	6.28
X_1X_2	−3.03	0.87	−3.50	0.0023	−4.84	−1.22
X_1X_3	1.81	0.87	2.09	0.0497	0.002	3.62
X_2X_3	1.06	0.87	1.22	0.2352	−0.75	2.87
X_1^2	−4.82	0.90	−5.33	<.0001	−6.68	−2.92
X_2^2	−4.26	0.90	−4.72	0.0001	−6.14	−2.38
X_3^2	−3.04	0.90	−3.38	0.0030	−4.92	−1.16

Table 3
Analysis of variance (ANOVA) for the experimental results obtained by using microwave assisted extraction.

Source	Sum of squares	Degree of freedom	Mean square	*p*-value Prob > *F*
Model	888.7620	9	98.7513	<0.0001
Error	119.9950	20	5.9998	
C-Total	1008.7570	21		

confirms that the model is highly significant. The regression coefficient from experimental data and the adjusted one were reasonably close to 1, which indicated a high degree of correlation between the observed and predicted values.

The significance of each coefficient measured using *p*-value and *F*-value is listed in Table 2. Smaller *p*-value and greater *F*-value mean the corresponding variables would be more significant. The *p*-value of the model is less than 0.0001, which indicates that the model is significant and can be used to optimize the extraction variables.

Now it is interesting to examine in Fig. 5 that the interaction plots of extraction time, microwave power and water-to-raw material on mucilage extraction efficiency. As can be seen from Table 2, there is a significant interactions between extraction time vs. microwave power ($p = 0.0023 < 0.01$) and extraction time vs. ratio liquid-to-solid ($p = 0.0497 < 0.05$). By contrast, a no significant interaction is observed between microwave power vs. ratio liquid-to-solid ($p = 0.2352 > 0.05$).

3.5. Analysis of the response surface model and contour plots

The interactive effects of the independent variables and their mutual interaction on the efficiency of the mucilage extraction yield can also be seen on three dimensional response surface profiles of multiple non-linear regression models. The 3D plots and their respective contour plots are shown in Fig. 6. They show the type of interactions between two tested variables and the relationship between responses and experiment levels of each variable, while keeping the other independent variable at its zero level. In the present study, the response surface and contour plots (Fig. 6) were obtained using Box–Behnken design (BBD).

Fig. 6A depict the effect of extraction time (X_1) and Microwave power (X_2) on the extracted mucilage yield, when the ratio of water to raw material was fixed at level 0. The mucilage recovery increase highly significantly ($p < 0.01$) with increasing the microwave power and the extraction time. After obtaining the highest extraction yield related to the time-power exposure; excessive time-power exposure under the microwave fields, leads to a decrease in mucilage extraction, due to a degrade of the polysaccharide molecules [14]. The recovery of mucilage mainly depends on the extraction time and microwave power as its quadratic and linear effects were highly significant ($p < 0.01$), confirming the single-factor experiment results (Table 2).

Fig. 6B shows the effect of extraction time (X_1) and ratio of water to raw material (X_3) on the mucilage recovery. Both extraction time and ratio water/raw material had positive effects on the response. It indicates a significant mutual interaction between extraction time (X_2) and ratio water/raw material (X_3). As the ratio of water to material reached to a high level, the mucilage yield increased with rising the extraction time. The weakest recovery is given at 1 min extraction time/500 W power level. The graph clearly shows that the mucilage yield increases by increasing the ratio water/raw material, due to the rise of diffusivity of the solvent into cells and enhance the desorption of the polysaccharides from the cells as explained below. Same behavior is observed for the extraction time at the fixed power followed by a decrease of mucilage recovery after an optimum yield, due a polysaccharides degradation, induced by an excessive time exposure.

Fig. 6C shows that with an increase of power, the extraction recovery of mucilage increased gradually, followed by a decline with further increase of water/raw material. This trend could be

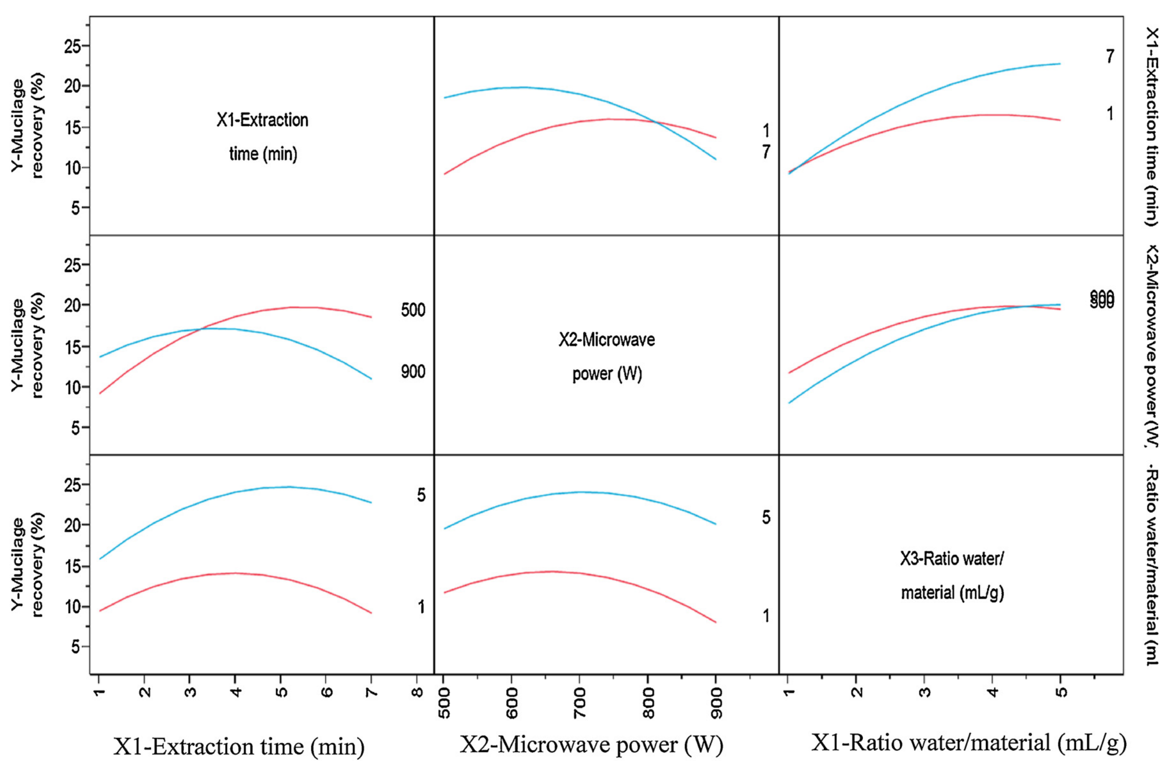

Fig. 5. Interaction plots of the experimental factors on the mucilage extraction efficiency.

attributed to increase in ratio water/raw material that decelerated mass transfer resulting from the lower heating efficiency under microwave conditions and the solubility of compounds [15].

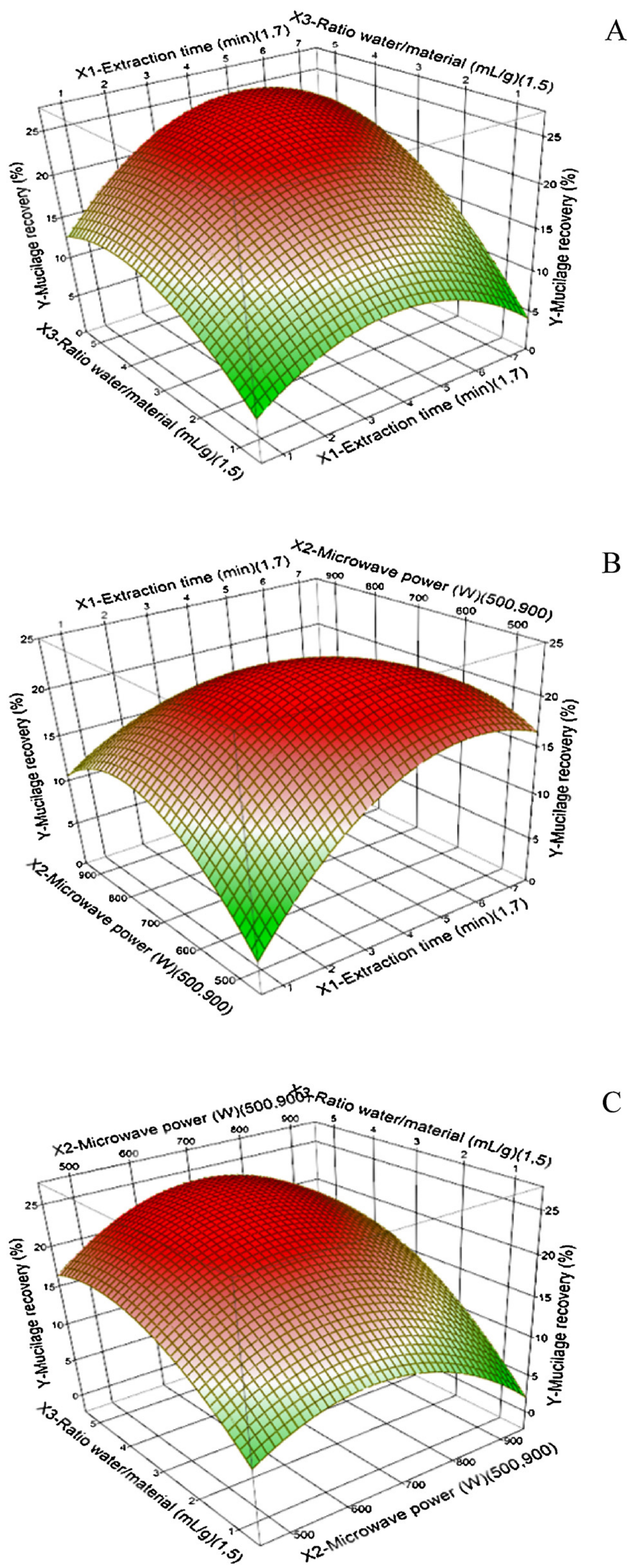

Fig. 6. Response surface analysis for the mucilage yield from *Opuntia ficus indica* cladodes with microwave assisted extraction method with respect to irradiation time (X_1); microwave power (X_2) and water-to-material ratio (X_3), according to the RSM model.

Table 4
Optimum conditions predicted and experimental value of response under those conditions.

X_1-Extraction time (min)	X_2-Microwave power (W)	X_3-Ratio water/ material (mL/g)	Experimental predicted
5.15	700	4.83	25,6 24.89

3.6. Validation of the developed models

Through these 3D plots and their respective contour plots, the final step of the RSM after selecting the optimum parameter combination is to predict and verify the improvement of the performance characteristics with the selected optimum parameters. In this work, after determining the optimum conditions and predicting the response under these conditions, a new set of experiment was designed and conducted with the selected optimal conditions of the process parameters to predict and verify the accuracy of the mathematical model. The results are shown in Table 4, the strong correlation between the actual (experimental) and predicted results confirm the effectiveness of the response surface models to reflect the expected optimization.

In view of validating the model, we selected all the experimental points and compared the experimental responses with the predicted values (Fig. 7). This graph proves that the second-degree model is quite realistic since it shows that the discrepancy between experimental and predicted values is very small.

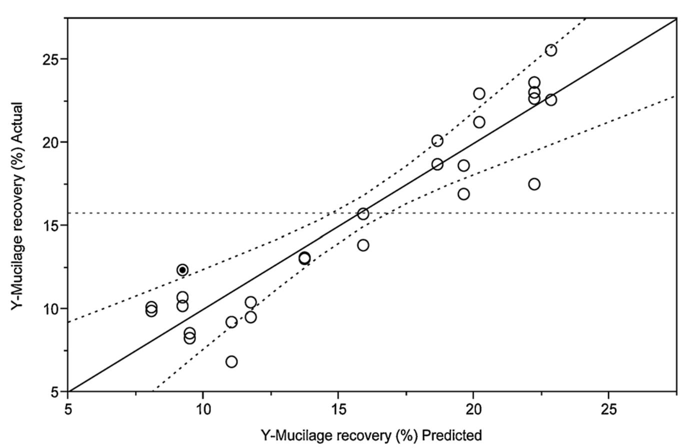

Fig. 7. Comparative plot between predicted and actual values of the mucilage recovery.

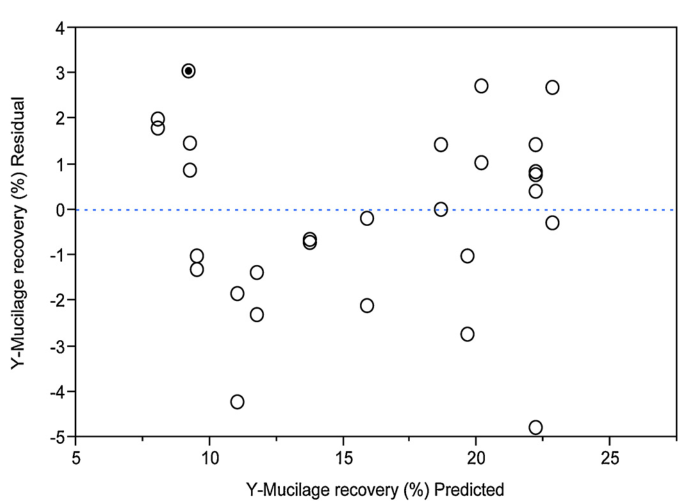

Fig. 8. A residual plot for microwave assisted extraction method obtained by RSM.

Furthermore, the adequacy of the model has been investigated also by the examination of residuals. Analysis of residuals appears to be a very useful and remarkably simple tool in model building and model criticism. The residuals play an important role in judging model adequacy. The residuals from the TPC regression model are shown in Fig. 8, which presents plot residuals (e) versus the predicted response ($\acute{y}$). A random pattern on these plots would indicate model adequacy [29].

4. Conclusion

The capability of mucilage extracted from water solution and the effects of processing parameters were investigated using RSM based on BDD. The most important reveals from this investigation are as follows:

- The optimum conditions were found as extraction time of 5.15 min, 700 W microwave irradiation and 4.83 water-to-powder ratio, and the optimum mucilage recovery of 25.6%;
- The results of RSM models based on validation data showed that RSM ($R^2 = 0.986$) is useful and perfect methods to predict mucilage recovery by applying MAE process;
- The research findings for MAE optimization with RSM model will provide effective guidelines and the results would be a good database to the Food hydrocolloid industry applications for use in health-care food;
- The leaves of *Opuntia ficus indica* Cladodes are rich in mucilages. However, further studies concerning the nutritional and health benefits are required before a large scale utilization of the *Opuntia* Cladodes can be recommended.

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