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Optimization of Ultrasonically Assisted Extraction of Pectin from Sugar Beet Pulp

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ABSTRACT: The Box-Behnken design combined with response surface methodology was used to optimize ultrasonic assisted extraction (UAE) of pectic substances from sugar beet pulp. The results indicated a highest extraction yield (16.3%) of pectic substances using UAE method using a frequency of 20 kHz and intensity of 99% for 10 min. In parallel, conventional acid extraction of pectic substances was also carried out. The highest yield could obtain by conventional method was 16% at a temperature of 90°C for 4 h. The crude extract of both procedure was then purified by centrifugation. The color attributes of UAE pectin compared to that of extracted by conventional method.

Industrial relevance: Pressed sugar beet pulp, a residue of the sugar processing industry, is sold as animal feed at very low prices and readily available for revalorization, with few other commercial uses. Moreover, the drying process requires high energy and often presents an environmental problem. Therefore, numerous attempts have been made to utilize this waste as a value-added product. However, so far no attempts have been made to investigate the ultrasonic assisted extraction (UAE) of sugar beet pulp pectin (SBPP) and evaluate their color attributes. In this work, a three variable, three level experiment Box-Behnken design based on respond surface methodology was used to optimize ultrasonic assisted acid-extraction of pectic substances from sugar beet pulp. The optimized results showed that the highest extraction yield of pectic substances by ultrasonic assisted extraction could be achieved at 16.26%, using ultrasound frequency of 20.2 kHz and ultrasound intensity of 99% for 10 min. The suitability of the model equation for predicting the optimum response value was treated using the selected optimal condition. The predicted extraction yield of pectic substances was 16.26%, which was almost consistent with the conventional extraction yield of pectic substances of 16.01%. UAE pectin had lower redness (a*) and yellowness (b*) values than its analogous ($p<0.05$), while the lightness (L*) value was not significantly different ($p>0.05$).

Keywords: Ultrasonic Assisted extraction (UAE); Response Surface Methodology (RSM);Sugar Beet Pulp Pectin (SBPP).

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) pulp (SBP), a residue of the sugar processing industry, is sold as animal feed at very low prices and readily available for revalorization, with few other commercial uses. Moreover, the drying process requires high energy and often presents an environmental problem. Therefore, numerous attempts have been made to utilize this waste as a source of pectin (Phatak *et al,* 1988; Rombouts and Thibault, 1986), dietary fibers (Michel *et al.*, 1988), a biosorbent for the removal of heavy metal ions (Reddad *et al.*, 2002) and biofuels (Zheng *et al.*, 2012). SBP has a high pectin content (15-30%) compared to pectin obtained from other sources such as citrus, apple and sunflower pectins, Sugar beet pectins have the advantage that the raw material is already dried (Mata *et al.,* 2009). Specifically, a focus has been placed on pectin which exhibits superior emulsifying properties compared with commercial pectins (Ma *et al.,* 2013).

As an applicable and new method, the application of UAE technique from plant material is widely published (Leonellia and Masonb, 2010); however, a few experiments have been done for pectin extraction using this innovative method. All of the published studies in application of ultrasound indicate increased yield or extraction rate as well as reduction in extraction time.

The effect of ultrasonic treatment on pectin stability in aqueous solution was studied to establish conditions minimizing degradation (Panchev *et al.,* 1988). Ultrasonication improved the pectin yield by 28% and reduced the extraction time. The effect of high-intensity ultrasound on the rheological and optical properties of high methoxyl pectin dispersions was studied by Seshadri *et al.* (2003). Intensifying the process of pectin de-esterification by combining acid maceration of raw materials with ultrasound treatment was also investigated (Panchev *et al.,* 1994). In this study, an additional decrease in the degree of esterification of pectin and a significant increase in the yield of pectin (ca. 18%) were reported. The optimum ultrasound treatment proved to be within 24-30 min and prolonged treatment can result in obtaining pectin with lower gel strengths (Panchev *et al.,* 1994). In our study, ultrasonic assisted extraction parameters were optimized from sugar beet pulp via response surface methodology (RSM) with a three-variable-three-level Box Behnken design (BBD), which could maximize the yield. The further purpose was to compare functional properties of pectin extracted by conventional and UAE methods.

MATERIALS AND METHODS

Pressed SBP (*flores* variety) was supplied by Khoy sugar factory (Khoy, Iran). It was oven dried at 40°C for 24 h and pulverized into powder to go through 60-mesh sieve using a desktop hamermill (Glencrestone Ltd.) and stored at room temperature. Food grade corn oil was purchased from local market used to prepare emulsions. Analytical grade anhydrous ethanol and hydrochloric acid (HCl) and analytical grade 25% (W/W) ammonia solution (NH3.H2O) were purchased from "Dr Mojallali Chemical Complex Co." (Tehran, Iran).

A. Ultrasonic Assisted Extraction from sugar beet pulp

The extraction of pectic substances from the dried sugar beet pulp(SBP)with 2% of moisture was carried out according to the experimental design shown in Table 2.

Table 1: Code and level of Independent variables in the response surface design.

Name	Unit	Low	Center	High
Frequency(X_1)	kHz	-1 (20)	0 (40)	1 (60)
Time(X_2)	min	-1 (10)	0 (20)	1 (30)
Intensity(X_3)	%	-1 (60)	0 (80)	1 (100)

Table 2: Box-Behnken design and observed responses.

Run	Independent variables			Response [pectic substances yield (%)]
	X_1 Frequency(kHz)	X_2 Time(min)	X_3 Intensity (%)	
1	40(0)	10(-1)	60(-1)	14.4
2	20(-1)	10(-1)	80 (0)	14.7
3	20(-1)	20(0)	60(-1)	13.6
4	40(0)	30(1)	60(-1)	14.0
5	40(0)	20(0)	80(0)	14.6
6	40(0)	30(1)	100(1)	15.5
7	60(1)	20(0)	100(1)	14.8
8	40(0)	20(0)	80(0)	14.3
9	40(0)	20(0)	80(0)	14.3
10	40(0)	20(0)	80(0)	14.0
11	60(1)	20(0)	60(-1)	14.6
12	60(1)	10(-1)	80(0)	14.5
13	20(-1)	20(0)	100(1)	15.7
14	60(1)	30(1)	80(0)	14.9
15	40(0)	20(0)	80(0)	14.8
16	40(0)	10(-1)	100(1)	15.8
17	20(-1)	30(1)	80(0)	14.2

The solid-liquid ratio in the suspension samples was maintained at 1:20 (w/v). These suspension samples were stirred at 200 rpm with a digital mechanical agitator (Heidolf, 2000, Germany). The pH of the suspension was adjusted to pH=1 with 12M HCL. Ultrasound treatments were carried out with a probe ultrasonic processor (Tapered tip: KE76, BANDELIN SONOPULS, Germany). Probe type ultrasonic device was operated at an amplitude of 10-100%, maximum admissible amplitude setting 72%, frequencies of 2, 4, 6, 8 (cycle×10%), maximum time of 99 min, the length and diameter of horn Tapered tip was 6 mm and 135 mm, respectively. After UAE processing of SBP the slurries were cooled down, adjusted to pH of 4.5 with 25% (W/W) ($NH_3.H_2O$) and filtered using a Buchner funnel under vacuum conditions. The supernatant recovered and the volume was measured. After using two volumes of 100% ethanol for 1 h at ambient temperature. The pectin was precipitated and recovered, then hand-squeezed in a nylon cloth to remove the ethanol. Finally, the sugar beet pulp pectin (SBPP) was washed twice with 100% ethanol, hand-squeezed in a nylon cloth to remove the residue of ethanol and dried in a hot air oven at 50 °C for 5 h.

B. Extraction of pectin from sugar beet pulp by conventional method

On the basis of preliminary experiments, proper ranges of extraction pH, ultrasonication time and ultrasound frequency were chosen in order to obtain the maximum yield of pectin. To extract the pectin with conventional method, similar protocol of UAE applied, except that instead of ultrasound treatment in extracting stage, a water bath heating was used at a constant temperature of 90 °C for 4 h was performed.

C. Pectin yield

The yield of SBPP was calculated as:

$$\text{Yield of SBPP}(\%)=\frac{m_0}{m}\times 100\%$$

Where m_0 (g) is the weight of dried SBPP, m (g) is the weight of dried SBP powder.

D. Pectin purification

The extracted pectins were further purified by centrifugation process. Dried pectins were dissolved in fresh Milli-Q water (solid-liquid ratio;1:200,w/w) for 15 h and centrifuged at 30000 g for 20 min at room temperature using a centrifuge (Heraeus Christ Labofuge II, Germany) to remove the water insoluble fraction (WIF). The supernatant was then filtered through both 11 and 3µm Millipore membranes. The filtrates were dried in a hot-air oven at 40°C for 5 h and weighed. Obtained pectins were used for characterization (Yapo *et al*, 2007).

E. Color measurements

The color of the purified pectin powders extracted with two above mentioned methods was evaluated using a Hunterlab colorimeter (model Miniscan XE), working with D65 (day light), and a measure cell with an opening of 30 mm, being used the CIE Lab colour parameters: L* from black (0) to white (100) ; a* from green (-) to red (+); and b* from blue (-) to yellow (+) (Kunte *et al.*, 1997). All tests were performed in triplicate. Analysis of variance was performed and results were evaluated by Independent Samples T-Test using the statistical software SPSS 16.0.

F. Experimental design

Based on the results of preliminary experiments, Ultrasound Frequency (kHz,X_1), Ultrasound Time (min,X_2) and Ultrasound Intensity (percent, X_3) were the independent variables selected to be optimized on the acid extraction of SBPP.

A three-variable-three-level BOX-BEHNKEN design (BBD) (software Design-Expert 7.0.1, Stat-Ease, Inc, Minneapolis, US) was employed in this optimization study (Wang *et al*, 2008, Yu *et al*, 2007). Extraction yield (Y) was taken as the response of the design experiment.

The whole design consisted of 17 experimental points carried out in random order. Five replicates (treatments 5, 8, 9, 10, 15) at the center of the design were used for estimating of a pure error sum of squares. The coded and uncoded (actual) levels of the independent variables are presented in Table 1.

A second-order polynomial Regression model was performed to express the yield as a function of the independent variables as follows in Eq. (1):

$$Y=\beta_0+\sum_{i=1}^{3}\beta_i X_i+\sum_{i=1}^{3}\beta_{ii}X_i^2+\sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j \quad \text{(Eq. 1)}$$

Where, Y represents the response variables, β_0 is a constant, βi , β_{ii} and β_{ij} are the linear, quadratic, and interactive coefficients, respectively. X_i and Xj are the levels of the independent variables($i\neq j$).

RESULTS AND DISCUSSION

A. Fitting the model

Table 2 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table3.

The data showed a good fit with Eq. (1), which were statistically acceptable at p‹0.05 level and adequate with satisfactory R^2 value. The full model filled Eq.(1)was made three-dimensional and contour plots to predict the relationships between the independent variables and the dependent variables. The operational parameters were optimized using Box-Behnken design combined with response surface methodology. The three parameters, ultrasonication time, ultrasound frequency and ultrasound Intensity were involved in the optimized experiment according to the Box-Behnken design. Table 2 represents the experiment design and corresponding response data. The regression coefficients of linear, quadratic and interaction terms of the model were calculated using the least square technique and are shown in Table 3.

Table 3: Estimated regression coefficients for the quadratic polynomial model and the analysis of variance(ANOVA) for the experimental results.

Parameter[a]	DF[b]	Sum of squares	F
linear			
X_1(Frequency)	1	0.034	0.50^{ns}
X_2(Time)	1	0.095	1.41^{ns}
X_3(Intensity)	1	3.45	51.25^{***}
Quadratic			
X_1^2	1	7.427×10^{-3}	0.11^{ns}
X_2^2	1	0.18	2.64^{ns}
X_3^2	1	0.41	6.04^{*}
Interaction			
$X_1 X_2$	1	0.18	2.69^{ns}
$X_1 X_3$	1	0.93	13.85^{ns}
$X_2 X_3$	1	1.000×10^{-4}	$1.487\times10^{-3\ ns}$
Model	9	5.30	8.76^{ns}
Lack of fit	3	0.12	0.46
Pure Error	4	0.35	
Cor total	16	5.77	
Correlation coefficient (R^2)	0.9185		
C.V.%	1.77		

[a]Coefficients refer to the general model
[b] Degree of freedom
***Significant at p<0.001
** Significant at p<0.01
* Significant at p<0.05
ns Not significant

The data presented in Table 3 show that; one linear(X_3) and one quadratic parameter (X_3^2) were significant at the level of p<0.001 orp<0.01. The coefficients 0f independent variables determined for the second order polynomial model are shown in the following equation:

$$Yield=14.41+0.66\ X_3-0.48X_1.X_3+0.31X_3^2 \qquad \text{Eq. (2)}$$

The analysis of variance (ANOVA) result of the model is shown in Table 3 including a good model performance with the correlation coefficient (R^2) value of 0.9185. The calculated model was able to explain 91.85% of the result in the pectin extraction of sugar beet pulp (Wang et al., 2008). The statistical analysis gave high significant level (p=0.0046) at testing the goodness of fit of the mode in pectic substances extraction. F-value for the lack of fit was insignificant (p=0.7241), thereby confirming the validity of the model. The value of coefficient of variation (C.V.) was 1.77% suggested that the model is reproducible (Wanasundara and Shahidi, 1996). The results indicated the model could work well for the prediction of pectin extract from sugar beet pulp.

B. Comparison of UAE with conventional method

Two selected solutions of UAE parameters are shown in Table 4. The selected solution class by RSM in the case of UAE parameters was a frequency of 20.21 kHz, time of 10 min and intensity of 99.9% which had the highest yield of pectic substances (16.26%). While, in the case of conventional method the highest yield of pectic substances with an extraction temperature of 90°C for 4 h at pH of 1 was 16.01%.

Table 4: Selected solution of Ultrasound Assisted Extraction (UAE) Factors.

Class	Desirability	Frequency	Time	Intensity	Yield%
1	1	20.21	10.00	99.90	16.26
2	1	24.26	10.00	99.17	16.09

Conventional method is very time consuming and energy intensive. It takes hours for complete extraction of pectic substances from raw material, whereas UAE provides a viable alternative.

Ultrasound can assist extraction processes both through cell disruption and by enhancing mass transfer in the boundary layer surrounding the solid matrix. This was documented by the improved method for extraction of pectin from apple pomace. Extraction of pectin was carried out in concentrated nitric acid using 22 kHz intermittent ultrasonication at 1-1.2 Wm^{-2} (30-60 min) followed by coagulation with 95% ethanol and separation by filtration. Ultrasonication improved the pectin yield by 28% and reduced the time for extraction (Panchev *et al.*,1988). The mechanical effects of ultrasound provide a greater penetration of solvent into cellular materials and improve mass transfer. Combined with this effects, the effects of micro streaming enhance mass transfer; which results in more efficient method for extraction. Similar results were reported that the yield of medicinal compounds such as helicid which is normally extracted by refluxing in ethanol can be obtained in a 50% higher yield in half the extraction time at room temperature using ultrasound (Yu *et al.*,1991).

C. Analysis of response surface

Response surface methodology (RSM) was used to determine the optimum condition and the integral effects of three independent variables. The best way of explaining the influence of process variables on the pectic substances yield is to generate surface response plots of the model. Fig.1 shows the surface plot for the optimization condition of ultrasond-assisted extraction of pectic substances fromSBP.

In this work, 3D Response surfaces were obtained by keeping on the variables constant at zero level, while varying the other two variables. The response surfaces showing the effect of ultrasound Frequency (X_1) ultrasonication time (X_2) on the yield of pectic substances as represented by Eq.(2) are shown in Fig.1a. The effect of ultrasound intensity on the yield has been assumed to be zero in this instance.

As can be seen from this figure, the yield of pectic substances mainly depend on the ultrasound frequency. This figure shows an interactive increase on the yield response as the ultrasound frequency decreases. The increase in pectic substances yield is interactive as a function of frequency for all the ultrasonication times tested corresponding to Eq.(2). It implied that the time is less significant than other two effects.

Fig.1b shows the effects of ultrasonication time (X2) andultrasound intensity(X3) on the yield of pectic substances according to Eq.(2). The effect of ultrasound frequency is set at zero level in this instance. As shown in fig.1b, ultrasound intensity have positive impact on pectic substances yield. However, the relationship between the extraction yield and the time of ultrasonication in the range of the times tested was found to be non-significant ($p>0.05$).

The linear, quadratic and interactive relationship between extraction yield and ultrasound intensity holds at all times tested. It implied that increase in extraction yield mainly rely on the ultrasound intensity and ultrasonic intensity play a key role in extraction efficiency. The ultrasound frequency exerts a negative impact on the yield response; hence the pectic substances yield increase interactively with the decrease in the ultrasound frequency within a range of 20-60 kHz. These observations are in agreement with earlier report, where the yield of rutin from Chinese Scholar Trees using ultrasound process at 20 kHz result in 20% yield increase in 30 min (Paniwynk *et al*, 2001).

Fig.1c illustrates the effects of ultrasound frequency(X_1) and intensity(X_3) on the yield of pectic substances calculated by Eq. (2) at fixed ultrasonication time of 20 minutes.

Fig.1c shows a sharp increase in yield with increase in ultrasound intensity, whereas yield decreased with increase in frequency. The highest yield of pectic substances is obtained when frequency was at its lowest and intensity was at its highest within the time range tested.

D. The color attributes

In order to evaluate how extraction method influences the color of purified pectin extracted from SBP, lightness(L*), redness (a*) and yellowness(b*) of the pure pectin were measured. The experimental L*, a* and b* mean value are shown in Table 5.

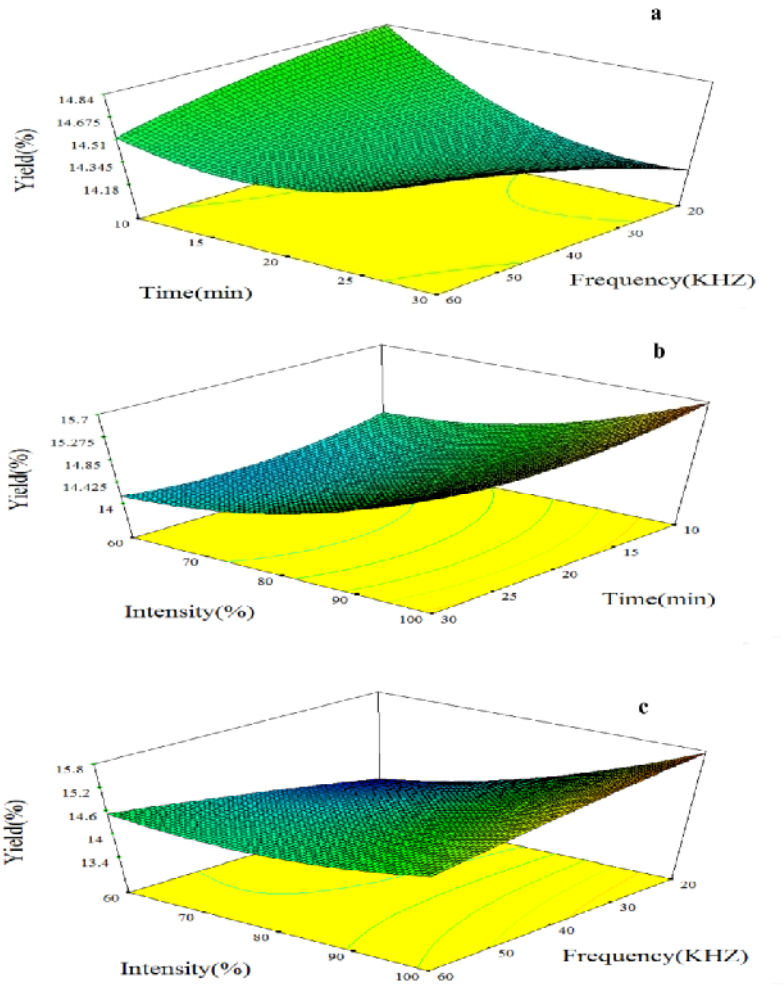

Fig.1. Response surfaces for the yield of SBPP: (a) Effect of time and ultrasound frequency on the yield at a constant ultrasound intensity (80%); (b) Effect of time and ultrasound intensity on the yield at a constant ultrasonic frequency (40kHz); (c) Effect of ultrasound frequency and intensity on the yield at a constant time (20min).

Table 5: Hunter lab color parameter values of pure pectin extracted from sugar beet pulp*.

Type of extraction	Hunterlab colour parameters		
	L*	**a***	**b***
UAE	41.20[a]	-2.50 [b]	10.40 [b]
Conventional	42.50[a]	0.23[a]	18.70 [a]

* Data are mean of triplicate measurements. Values in the same column with different superscript letters are significantly ($p<0.05$) different.

Conventionally extracted pectin had significantly ($p<0.05$) higher redness (a*) and yellowness(b*) values than those of UAE obtained pectin, whereas the lightness (L*) value of both pectins were the same. Pectin that extracted by ultrasound assisted method had a white appearance like that one extracted by conventional method.

CONCLUSIONS

The optimized results showed that the highest extraction yield of pectic substances by ultrasonic-assisted extraction could arrive 16.26%, using ultrasound frequency of 20.21 kHz and ultrasound intensity of 99.9% for 10 min. The suitability of the model equation for predicting the optimum response value was treated using the selected optimal condition.

The predicted extraction yield of pectic substances was16.26%, which was almost consistent with the conventional extraction yield of pectic substances of 16.01%.

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