Available online at www.elixirpublishers.com (Elixir International Journal)

Chemical Engineering

Elixir Chem. Engg. 66 (2014) 20987-20996

ISSN: 2229-712X

Optimization of microwave-assisted extraction of carbohydrates from corn Pericarp

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ARTICLE INFO

Article history:
Received: 9 May 2013;
Received in revised form:
20 January 2014;
Accepted: 25 January 2014;

Keywords
Microwave assisted extraction,
Response Surface Methodology,
Corn pericarp,
Carbohydrate.

ABSTRACT

Microwave-assisted extraction (MAE) was employed for production of carbohydrates from corn pericarp which is a waste of corn starch production by using hot compressed water as a solvent. Carbohydrates consist of glucose, xylose, arabinose and hemicellulose, while residues were composed of cellulose. By increasing the heating temperature, the solubilization rate increases and reached the value of 75.2% at 220 °C. In order to increase the carbohydrate yield the four independent variables such as heating temperature, come-up time, heating time and solid to liquid ratio were optimized by using the response surface methodology techniques. It includes fractional factorial design, the path of steepest ascent and central composite design. Subsequently, we have applied 2-step of experimental design including fractional factorial design and central composite design for accurate prediction of the optimum condition of MAE of carbohydrates from corn pericarp. In this paper, the total recycle of the corn pericarp have been done by investigating the detailed effects of microwave irradiation on chemical components in corn pericarp. The maximum yield of carbohydrates is about 70.8 % with predominant production of xylo-oligosaccharides.

Introduction

Corn pericarp

Corn pericarp is mainly composed of carbohydrates which include cellulose, hemicellulose and residual starch. Hemicellulose contains arabinoxylan (corn fiber gum). Corn arabinoxylan is mainly consist of a backbone of β-(1→4)-linked d-xylose with side chains of α-L-arabinose, 4-*O*-methylglucuronic acid and a trisaccharide composed of xylose, arabinose and galactose . Little phenolic acids such as ferulate and diferulate also bounded to arabinoxylan and contribute to cross-linkage between xylan chains and lignin chains.

In general, xylan and xylo-oligosaccharides have been reported to possess various bioactivities, such as lowering blood cholesterol level, reduction of stomach ulcer lesions, improvement of mineral absorption, regulation of lipid metabolism, decrease in the risk of colon cancer and modulation of the immune system etc.

Arabinoxylan has been extracted by various methods, including alkaline and acid hydrolysis , steam fractionation and hot compressed water as a solvent.

Response surface methodology

Response surface methodology (RSM) [18] is a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the Objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). The application of RSM to design optimization is aimed at reducing the cost of expensive analysis methods [10]. (e.g. Finite element method or CFD analysis) and their associated numerical noise.

Microwave-assisted extraction

Microwave irradiation was performed by using microwave oven using a Monobloc High-pressure Digestion Rotor (HPR-100). Corn pericarp (0.5–1 g) was suspended in 20–30 ml distilled water in the HPR-100 and mixed with a stirrer bar for 15 min to give sufficient penetration of water [4].Then reactant was heated to desired temperatures (140–220 °C) in 2–4 min and held at the temperature for 1–26 min with microwave irradiation. After irradiation, the product was immediately cooled in an ice bath to room temperature and filtrated to separate Solubilized fraction and residues. Solubilized fraction was preserved at −30 °C until experiments, and residues were freeze-dried.

Analysis of solubilized fraction

The effects of heating temperature on solubilization of carbohydrates, polyphenol and solubilization rate is determined by performing Microwave irradiations at 140–220 °C

Solid to liquid ratio → 1/30 (g/ml)
come-up time → 2 min
Heating time → 5 min.

The solubilization rate was determined by the following equation: Solubilization rate % = (wt of raw material) – (wt of residue) * 100 / (wt of raw material)

Neutral sugar composition analysis

After microwave irradiation, Seaman method was employed to hydrolyze the raw material and each fraction. High-performance anion exchange chromatography (HPAEC) on a Dionex DX-500 system with pulsed amperometric detector (ED-40) is used to analyse the monosaccharide composition by using 1.0 mm NaOH as a mobile phase[19].

Size Exclusion Chromatography (SEC)

To determine the Molecular weight of Solubilized materials, SEC on a column of MCI GEL CK04SS at 80 °C with refractive index detector was used. Eluent was deionized water and flow rate was 0.3 ml/min. The charged contaminants were eliminated through the purification of Solubilized materials, by passage through a joint column of anion and cation exchange resins.

Scanning Electron Microscope (SEM)

The investigation of effects of heating temperature on morphological properties of residues after MAE was employed

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by low voltage scanning electron microscope at 1.7 Kv on an amorphous carbon stage.

Experimental design and data analysis

2-step experimental design was used in this work to increase the yield of carbohydrates [21]. The predictor variables were coded by the following equation:

$x_i=(X_i-X_{i,0})/\Delta X_i$

Where,

x_i → coded value of independent variables

X_i → real value of the independent variable

$X_{i,0}$ → real value of the independent variable at the center point

ΔX_i → step change value.

The qualities of fitted models obtained by experimental data were examined by the coefficient of determination R^2. The analyzation of experimental results was done by using the Least-Square Method (LSM). The regression model was analyzed by using Analysis of Variance (ANOVA).

Fractional factorial design

At a condition far away from the optimum condition, FFD was constructed with eight experimental runs and three replications at the center point of the design.

The following regression equation [18,21] was obtained by the LSM:

$$Y = \beta_0 + \sum_{i=1}^{K} \beta_i x_i + \sum_{i=2}^{K} \beta_{1i}\, x_1\, x_i$$

Central composite design

Finally, the central composite design (CCD) was performed to determine the optimum condition. CCD was designed with five replications at the center points, with four axial points and four star points (±á). The following regression equation [21] was fitted to the response resulted from CCD by the LSM:

$$Y = \beta_0 + \sum_{i=1}^{K} \beta_i x_i + \sum_{i=1}^{K} \beta_{ii}\, x_i^2 + \beta_{ij}\, x_i\, x_j$$

Where,

Y → Estimated carbohydrates yield,

x_i → Coded value of an independent variables,

â's → Regression coefficients.

Adequacy of this model was confirmed by ANOVA.

Calculation of severity parameter

Without addition of acid catalyst severity parameter of hydrothermal treatment was calculated by following equation

$$\log R = \log\left[\int_0^t \exp\left(\frac{T(t) - Tref}{14.75}\right)\right](dt)$$

Where,

t → Reaction time (min),

T(t) → Temperature–time function for gradually heated processes (°C)

T ref → Reference temperature which was set to 100 °C

Materials And Methods

Corn pericarp was supplied from a corn starch manufacture. Preparation of a mixture of xylo-oligosaccharides with the degree of polymerization of 2–10 was done by partial hydrolysis of beech 4-*O*-methyl-glucuronoxylan with 0.1 N H_2SO_4 for 1 h at 92 °C followed by neutralization with barium carbonate and passage through a joint column of Dowex 50 × 8 (H^+) and Dowex 1 × 8 (aco).

Composition analysis

The following method which is given in the table 1 was used to analyse the powdered corn pericarp and their component.

Extraction and separation of hemicelluloses

Hot water extraction (prehydrolysis) for one hour at 180 ºC has been applied for selective removal of hemicellulose [1] from corn pericarp before chemical pulping processes. [5] There is a complete removal of the hemicelluloses from biomass and herbaceous materials without high degradation by using around 15 minutes hot liquid water extraction at 200-230 ºC[6,9]. Partial depolymerization and solubilization of the lignin can take place during this extraction. However, utilization of hot water alone cannot satisfy complete delignification. The dissolution of cellulose can only be achieved at higher water temperatures. The maximum solubilization of the cellulose (recovered as glucan) at 200-230 °C from biomass was around 22 %. The biomass fractionation is only possible by water steam at this temperature range. The water steam can dissolve the hemicelluloses but it cannot satisfy complete removal of lignin.

Higher hemicelluloses recovery and greater lignin removal could be obtained by passing hot water continuously through a stationary biomass. Generally, significant increase of hot water flow rate enhances the hemicelluloses removal. Pretreatment using hot liquid water can be a promising process to improve cellulose digestibility, sugar extraction, and pentosan recovery. In this type of pretreatment, the hemi-acetal linkages of hemicellulose are cleaved, O-acetyl and other acids are liberated from hemicelluloses. This allows to form and release acetic and uronic acids [2]. However, these acids are useful to catalyze removal of oligosaccharides from hemicellulose. The optimization of pre-treatment processes is usually based on highest overall sugar yield with minimum degradation of the carbohydrate component.

Pretreatment method

Extraction of hemicelluloses can be carried out in neutral or alkaline solutions. Hence, hemicelluloses are divided into two fractions: water-soluble and water-insoluble. Problems in carrying out water extraction of cereal bran xylans may occur due to hemicelluloses is bound to lignin or cellulose through ferulic acid bridges and also because of hydrogen bonding between the non-substituted xylose residues and the cellulose chains[20].

Several processes have been introduced for hemicellulose isolation from grain crops and from cereal brans, involving water and alkali extraction as well as other combinations such as alkali and hydrogen peroxide, alkali and chlorite solutions or dimethyl sulfoxide . In addition, pilot-scale isolation of cereal xylans has been demonstrated, indicating the feasibility of scaling up to an industrial level.

Only 20-40% (w/w) of cereal grain hemicelluloses is typically water-extractable. Water extraction allows the isolation of high molar mass hemicelluloses and helps preserve the hemicellulose structure although the resulting yields are relatively low. .Yields can be highly improved by extraction with other solvents, most commonly applied under alkaline conditions. Such treatments can cause deacetylation in the case of certain hemicelluloses so the original structure will not then be preserved. Selective arabinoxylan extraction, avoiding the co-isolation of β-glucan, can be performed with barium hydroxide solution contrary to sodium or potassium hydroxide solutions .

The flow sheet for extraction process was given in figure 1.

Comparison of different hydrolysis method was given in the table 2.

Microwave assisted extraction process

Microwave assisted extractor was given in figure 2.

Principle elements of a microwave device

Microwave generator: Magnetron, which generates microwave energy

Wave guide: This is used to propagate the microwave from the source to the microwave cavity

The applicator: Where the sample is placed

Circulator: This allows the microwave to move only in the forward direction.

Working principle

The destruction of organic matter in samples with high sugar content was done by microwave digestion which is appreciable when compared to conventional approaches. High pressure microwave assisted digestion (HPMW), is currently the most usually applied digestion technique which enables sample decomposition at high temperatures without significant losses of volatile elements, this procedure destroys the organic matter content of the sample completely by using concentrated acids. Organic components are oxidized; carbon and hydrogen are converted into Carbon dioxide and water. An important advantage is the high throughput, depending on the digestor rotor characteristics. Nevertheless, closed vessel decomposition has a significant drawback: these systems usually limit the sample amount to an only few grams of organic matter.

Microwave theory

Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz and positioned between the X- ray and infrared rays in the electromagnetic spectrum. In modern day science microwaves serves two major purpose – communication and as energy vectors. The latter application is the direct action of waves on materials that has the ability to convert a part of the absorbed electromagnetic energy to heat energy.

Microwaves are made up of two oscillating perpendicular field's i.e. Electric field and magnetic field. Unlike conventional heating which depends on conduction – convection phenomenon with eventually much of the heat energy being lost to the environment. Whereas in case of MAE, heating occurs in a targeted and selective manner with practically no heat being lost to the environment as the heating occurs in a closed system. This unique heating mechanism can significantly reduce the extraction time (usually less than 30 min) as compared to Soxhlet extraction.

The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field.

Heating is affected only at a frequency of 2450 MHz. The electric component of the wave changes 4.9 × 104 times per second. Every time the solvent molecules tries to align itself with the electric field to keep itself in the same phase, but with the electrical component of the wave changing at such a rapid speed, the molecules fails to realign itself and starts vibrating which generates heat through frictional force. With frequency greater than 2450 MHz the electrical component even changes at a much higher speed as a result the molecules does not get sufficient time to even start to align itself with the external field as a result no heating occurs. If the frequency is less than 2450 MHz the electrical component changes at a much lower speed and the molecules get sufficient time to align itself with the electric field, thus there occurs no heating.

The above mechanisms clearly indicate that only dielectric material or solvents with permanent dipoles only do get heated up under microwave. The efficiency with which different solvents heat up under microwave depends on the dissipation factor which is indeed the measure of the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecules.

Extraction principle

Even though dried plant material is used for extraction in most cases, but still plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect, evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell . The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptures cells to the surrounding solvent thus improves the yield.

This phenomenon can even be more intensified if the plant matrix is impregnated with solvents with higher heating efficiency under microwave. Higher temperature attained by microwave radiation can hydrolyze ether linkages of cellulose, which is the main constituent of plant cell wall, and can convert into soluble fractions within 1 to 2 min. The higher temperature attained by the cell wall, during MAE, enhances the dehydration of cellulose and reduces its mechanical strength and this in turn helps solvent to access easily to compounds inside the cell. In order to study cell damage during the MAE experiments, samples were examined by scanning electron microscopy [8].

Advantages of closed-vessel MAE system

→ Because of the increased pressure inside the vessel, the boiling point of the used solvents get increases so they can reach higher temperatures than open vessel systems, in turn the time needed for the microwave treatment get decreases.

→ During microwave irradiation loss of volatile substances is completely avoided.

→ Less solvent is required because no evaporation occurs, it is not necessary to add solvent continuously to maintain the volume. Also, the risk of contamination is avoided as a result there is little or no risk of airborne contamination.

→ The fumes produced during an acid microwave extraction are contained within the vessel, so no provision is needed for handling potentially hazardous fumes.

Results And Discussion

Composition Analysis Of Native Sample

The chemical composition (%, w/w, dry weight basis) and relative monosaccharide composition of raw materials were given in Table 3. Total content of xylose, glucose and arabinose accounted for 90.2% (w/w) showing abundance of arabinoxylan and cellulose as main polysaccharides.

Effect of microwave heating on corn pericarp

The effects of heating temperature on the solubilized fraction from corn pericarp were investigated by microwave irradiation at 140–220 °C under fixed solid to liquid ratio of 1/30 (g/mL), 2 min of come-up time and 5 min of heating time at the desired temperatures. [4] Solubilization rate, carbohydrates yield, polyphenol yield and uronic acid yield in soluble fraction were shown in Fig 3.[14] .

The solubilization rate increased with increase in heating temperature, and reached 75.2% at 220 °C (Fig. a). [16] Carbohydrates yield also increased with increase in heating temperature and attained 489 mg/g at 200 °C, however, decreased by heating at 220 °C due to secondary degradation of carbohydrates into furfural and other derivatives. Hemicellulose such as arabinoxylan is autohydrolyzed by hot compressed

water by heating above 180 °C and further heating leads to rapid secondary degradation .

Relative monosaccharide composition (% w / w) of residues and the Solubilized fraction after microwave irradiation was given in table 4.

From table 4 room temperature represents stiring for 15 min in H_2O without microwave irradiation. Optimum point represents treatment under the optimum microwave irradiation condition (175 °C at 18 min).

From table 4 we have observed in Solubilized fraction is Xylosed increased with increase in temperature attain 42.6% at 200° C.

Behaviour of solubilization of xylose and arabinose strongly correspond with that of carbohydrates yield. Carbohydrate yield and total content of xylose, arabinose showed good correlation.

In residue glucose level increases with increase in temperature and attain 95.7 % at 220 °C.

Optimization

Fractional factorial design

Independent variables of the FFD and the carbohydrates yield were given in table 5.

Residual

Residual (or error, or deviation) is the difference between the observed value y* of the dependent variable for the j^{th} experimental data point (x_{1j}, x_{2j}, …, x_{pj}, yj*) and the corresponding value y_j given by the regression function $y_j = b_0 + b_1x_j + b_2x_j + \dots b_pxp_j$. [10]

$$r_j = y_j^* - y_j \longrightarrow (1)$$

Parameters b (b_0, b_1, b_2, … b_p) are part of the ANOVA output . If there is an obvious correlation between the residuals and the independent variable x (say, residuals systematically increase with increasing x), it means that the chosen model is not adequate to fit the experiment. A plot of residuals is very helpful in detecting such a correlation. This plot will be included in the regression output if the box "Residual Plots" was checked in the regression input dialog window .

Standard residual

Standard (or standardized) residual is a residual scaled with respect to the standard error (deviation) S_y [10] in a dependent variable:

$$r_j' = r_j / S_y \longrightarrow (2)$$

The quantity Sy is part of the "Regression statistics" output. Standardized residuals are used for some statistical tests, which are not usually needed for models in physical sciences.

Coefficients

The regression program determines the best set of parameters b (b_0, b_1, b_2, … b_p) in the model $y_j=b_0 +b_1x_{1j}+b_2x_{2j} +\dots b_px_{pj}$ by minimizing the error sum of squares SSE. Coefficients are listed in the second table of ANOVA. These coefficients allow the program to calculate predicted values of the dependent variable y (y_1, y_2, … y_n), which were used above in formula (2) and are part of Residual output .

Sum of squares

In general, the sum of squares of some arbitrary variable q [10,11] is determined as:

$$SS_q = \sum_j^n (q_j - q_{avg})^2 \longrightarrow (3)$$

Where

q_j - jth observation out of n total observations of quantity

q_j - average value of q in n observations

$q_{avg} = (\sum_j^n q_j)/n$

In the ANOVA regression output one will find three types of sum of squares

1) Total sum of squares SS_T

$$SST = \sum_j^n (yj^* - y^*_{avg})^2, \longrightarrow (3a)$$

Where

$y^*_{avg} = (\sum_j^n y_j^*) / n$

It is obvious that SST is the sum of squares of deviations of the experimental values of dependent variable y* from its average value. SST could be interpreted as the sum of deviations of y* from the simplest possible model (y is constant and does not depend on any variable x):

$y = b_0$, with $b_0 = y^*_{avg}$

SST has two contributors

residual (error) sum of squares (SS_E) and regression sum of squares(SS_R):

$$SS_T = SS_E + SS_R \longrightarrow (5)$$

2) Residual (or error) sum of squares SS_E

$$SS_E = \sum_j^n (r_j - r_{avg})^2 \longrightarrow (6)$$

Since in the underlying theory the expected value of residuals r_{avg} is assumed to be zero [12], expression (6) simplifies to:

$$SS_E = \sum_j^n (r_j)^2 \longrightarrow (6a)$$

The significance of this quantity is that by the minimization of SS_E the spreadsheet regression tool determines the best set of parameters $b= b_0,b_1,b_2 \dots b_p$ for a given regression model. SS_E could be also viewed as the due-to-random-scattering-of -y*-about-predicted-line contributor to the total sum of squares SS_T. This is the reason for calling the quantity "due to error (residual) sum of squares".

3) Regression sum of squares SS_R

$$SS_R = \sum_j^n (yj - y^*_{avg})^2 \longrightarrow (7)$$

SS_R is the sum of squares of deviations of the predicted-by-regression-model values of dependent variable y from its average experimental value y*avg. It accounts for addition of p variables ($x_1,x_2 \dots x_p$) to the simplest possible model (4) (variable y is just a constant and does not depend on variables x),

i.e. $y = b_0$ vs. $y = b_0 + b_1x_1 + b_2x_2 + \dots + b_px_p$.

Since this is a transformation from the "non-regression model" (4) to the true regression model (1), SSR is called the "due to regression sum of squares".

$$SSR = \sum_i^p bi \sum_j^n (x_{i\,j} - x_{avg})\, y_j \longrightarrow (7a)$$

Where

$x_{avg} = (\sum_j^n xj^*)/n$

or

$$SS_R = \sum_i^p b_i \sum_j^n x_{i\,j}\, y_j^* - (\sum_j^n y_j^*)^2 / n \longrightarrow (7b)$$

Relationships (7a-b) give the same numerical result, however, it is difficult to see the physical meaning of SS_R from them.

Mean square (variance) and degrees of freedom

The general expression for the mean square of an arbitrary quantity q is:

$$MS_q = SS_q / df \longrightarrow (8)$$

SSq is defined by (3) and df is the number of degrees of freedom associated with quantity SSq. MS is also often referred to as the variance. The number of degrees of freedom could be viewed as the difference between the number of observations n and the number of constraints (fixed parameters associated with the corresponding sum of squares SSq).

1). Total mean square MST (total variance)

$$MS_T = SS_T/(n - 1) \longrightarrow (9)$$

SST is associated with the model (4), which has only one constraint (parameter b_0), therefore the number of degrees of freedom in this case is:

$$df_T = n - 1 \longrightarrow (10)$$

2). Residual (error) mean square MS_E (error variance)

$$MS_E = SS_E / (n - k) \longrightarrow (11)$$

SSE is associated with the random error around the regression model (1), which has k=p+1 parameters (one per each variable

out of p variables total plus intercept). It means there are k constraints and the number of degrees of freedom is :

$df_E = n - k$ ⟶ (12)

3). Regression mean square MSR (regression variance):

$MS_R = SS_R /(k - 1)$ ⟶ (13)

The number of degrees of freedom in this case can be viewed as the difference between the total number of degrees of freedom df_T (10) and

the number of degrees of freedom for residuals df_E (12) :

$df_R = df_T – df_E = (n - 1) - (n - k)$

$df_R = k - 1 =$ ⟶ (14)

Tests of significance and F-numbers

The F-number is the quantity which can be used to test for the statistical difference between two variances. For example, if we have two random variables q and v, the corresponding F-number is:

$F_{qv} = MS_q / MS_v$ ⟶ (15)

The variances MSq and MSv are defined by an expression of type (8). In order to tell whether two variances are statistically different, we determine the corresponding probability P from F-distribution function:

$P=P(F_{qv}, df_q, df_v)$ ⟶ (16)

Surface plot

This plot shows a three dimensional surface that connects a set of data points. A Surface chart is useful to find optimum combinations between two sets of data. Surface charts are useful to show how a variable (Z) changes according to two other variables (X and Y).Like a topographic map, the colors and patterns in a Surface chart indicate areas that contain the same range of values. Unlike other chart types, colors in a surface chart are not used to distinguish each data series. Instead, colors are used to distinguish the values.

Surface plot have been drawn for fractional factorial design which was given in figure 4.

Contour plot

A contour plot is a graphical techniques which is used to represent a 3 dimensional surface by plotting constant z slices, called contours, on a 2 – dimensional format.

Contour plot have been drawn for central composite design which was given in figure 5.

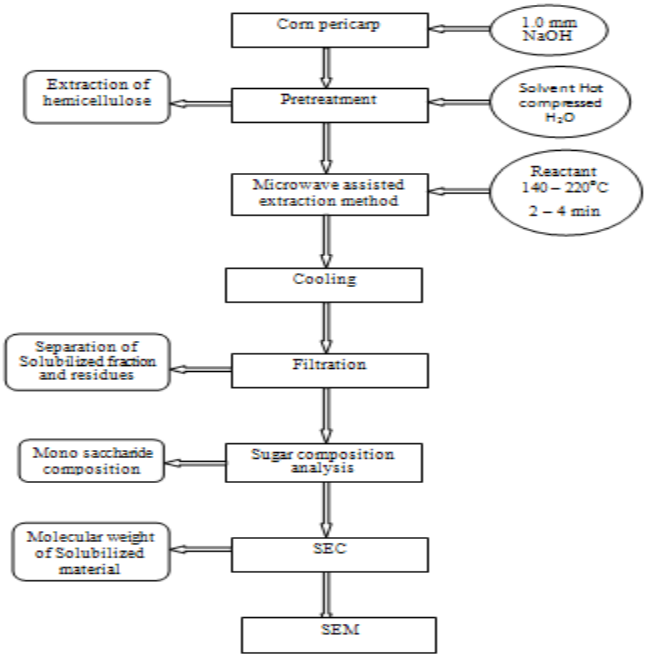


Fig. 1 Flow sheet for extraction process

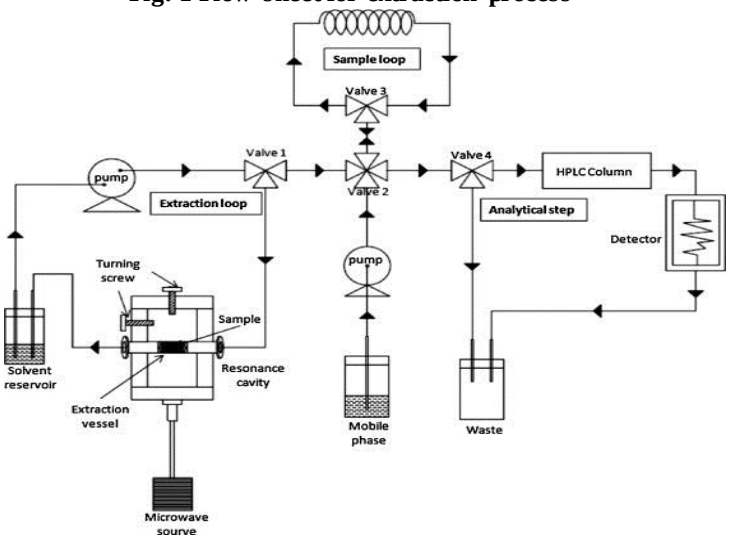


Fig. 2 Microwave assisted extractor

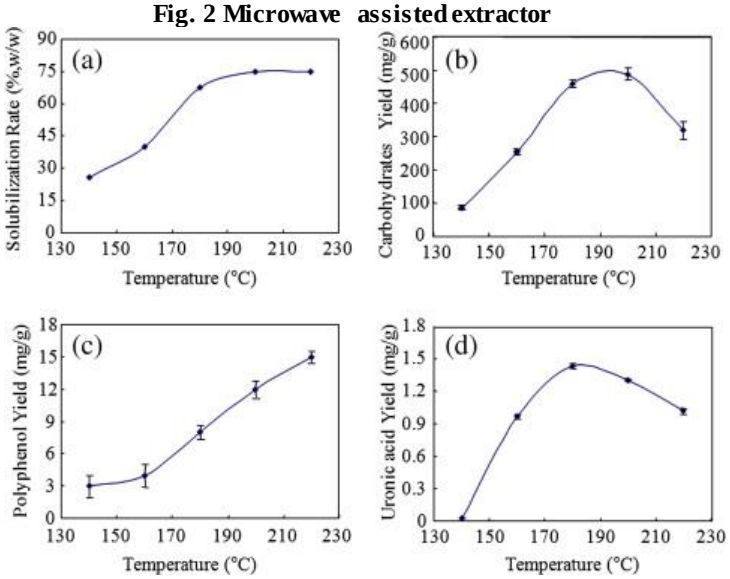
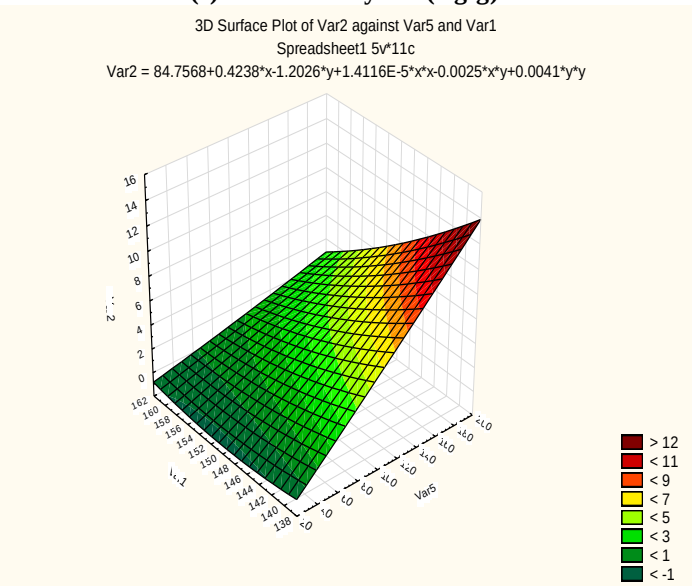


Fig.3 Effects of MAE on (a) solubilization rate (%, w/w), (b) carbohydrates yield (mg/g), (c) polyphenol yield (mg/g) and (d) uronic acid yield (mg/g).



Var 1 : X_1 , Var 2 : X_2 , Var 5 : Y

Fig. 4 Surface plot for fractional factorial design

ANOVA for screening regression model of FFD

Regression Statistics						
Multiple R	0.952852575					
R Square	0.907928031					
Adjusted R Square	0.884910038					
Standard Error	14.97042539					
Observations	11					
ANOVA						
	Df	SS	MS	F	Significance F	
Regression	2	17680	8840	39.44427543	7.18637E-05	
Residual	8	1792.9091	224.113636			
Total	10	19472.909				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-564.9090909	80.222359	-7.0417911	0.000108047	-749.9021813	-379.916
X Variable 1	4.1	0.5292845	7.74630707	5.50278E-05	2.879467835	5.320532
X Variable 2	23	5.2928447	4.34548933	0.002460152	10.79467835	35.20532
RESIDUAL OUTPUT						
Observation	Predicted Y	Residuals				
1	96.09090909	-4.0909091				
2	96.09090909	-9.0909091				
3	96.09090909	-12.090909				
4	32.09090909	9.9090909				
5	114.0909091	4.9090909				
6	32.09090909	16.909091				
7	114.0909091	-19.090909				
8	78.09090909	-6.0909091				
9	78.09090909	-8.0909091				
10	160.0909091	0.9090909				
11	160.0909091	25.909091				

ANOVA for regression model of FFD

Regression Statistics						
Multiple R	0.959565724					
R Square	0.920766379					
Adjusted R Square	0.867943966					
Standard Error	16.03594447					
Observations	11					
ANOVA						
	Df	SS	MS	F	Significance F	
Regression	4	17930	4482.5	17.43135753	0.00187147	
Residual	6	1542.909091	257.15152			
Total	10	19472.90909				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-584.4090909	89.23525335	-6.549083	0.000606328	-802.75989	-366.05829
X Variable 1	4.1	0.566956254	7.2315985	0.000354737	2.71270803	5.487292
X Variable 2	23	5.66956254	4.0567504	0.006674973	9.12708026	36.87292
X Variable 3	22	22.67825016	0.9700925	0.36946508	-33.491679	77.491679
X Variable 4	1	5.66956254	0.1763805	0.865797882	-12.87292	14.87292
RESIDUAL OUTPUT						
Observation	Predicted Y	Residuals				
1	96.09090909	-4.090909091				
2	96.09090909	-9.090909091				
3	96.09090909	-12.09090909				
4	36.59090909	5.409090909				
5	120.5909091	-1.590909091				
6	27.59090909	21.40909091				
7	107.5909091	-12.59090909				
8	84.59090909	-12.59090909				
9	71.59090909	-1.590909091				
10	155.5909091	5.409090909				
11	164.5909091	21.40909091				

ANOVA for regression model of CCD

Regression Statistics						
Multiple R	0.9784779					
R Square	0.9574191					
Adjusted R Square	0.9270042					
Standard Error	9.4552197					
Observations	13					
ANOVA						
	Df	SS	MS	F	Significance F	
Regression	5	14071.115	2814.22296	31.4785889	0.00011755	
Residual	7	625.80825	89.4011791			
Total	12	14696.923				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	38.022272	138.50573	0.27451768	0.79161194	-289.49174	365.5362821
X Variable 1	-0.04897	0.5448484	-0.0898776	0.93090209	-1.3373315	1.239392121
X Variable 2	-1.043636	0.9758381	-1.0694769	0.32034051	-3.3511268	1.263854166
X Variable 3	0.296176	5.8013253	0.05105317	0.96070944	-13.421779	14.01413065
X Variable 4	8.3246753	5.6001004	1.4865225	0.18073179	-4.917458	21.56680866
X Variable 5	0.9806061	0.1072652	9.14188206	3.8526E-05	0.72696411	1.234248034
RESIDUAL OUTPUT						
Observation	Predicted Y	Residuals				
1	502.86047	4.1395343				
2	526.96289	-4.96289				
3	510.64289	-4.64289				
4	524.38107	5.6189276				
5	531.06107	10.938927				
6	531.06107	-1.061073				
7	531.06107	-17.06107				
8	531.06107	3.9389274				
9	531.06107	2.9389274				
10	505.38107	5.6189276				
11	492.64289	-4.64289				
12	443.96289	-4.96289				
13	427.86047	4.1395343				

Table 1: Composition analysis

Component	Determination method
Moisture	Drying
Carbohydrate	Phenol sulfuric acid using a mixed std. Solution of solution of glucose, xylose, arabinose and galactose according to the composition determined after Seaman hydrolysis and High Performance Anion exchange chromatography (HPAEC)
Protein content	Elemental analysis and multiplied by 6.25 as a protein conversion factor
Lipid	Soxhlet extraction with 75% aqueous 1-propanol as an extracting solvent
Uronic acid	Method of Filisetti-Cozzi and Carpita [2]
Holo cellulose	Method of Uprichard [5]

Table 3: Chemical compositions (%, w/w) and relative monosaccharide composition (%, w/w) of corn pericarp sample (dry weight basis).

Component	Chemical composition
Moisture	8.0 ± 0.1
Carbohydrate	83.2 ± 3.2
Holocellulose	78.6 ± 0.5
á cellulose	25.1 ± 0.8
Acid insoluble lignin	4.0 ± 0.3
Protein	9.5
Lipid	6.6 ± 2.0
Uronic acid	0.6 ± 0.1
Ash	0.6

Table 2: Comparison of different hydrolysis methods [6]

Hydrolysis Method	Conditions	Glucose Yield (%)	Advantages	Disadvantages
Concentrated Acid	30-70 % H2SO4 T = 40 ℃ Time = 2-6 h	90	High sugar recovery High reaction rate	Environmental and corrosion problems High cost for acid recovery
Dilute acid	< 1 % H2SO4 T = 215 ℃ Time = 3 Min	50-70	High sugar recovery High reaction rate	Environmental and corrosion problems Sugar decomposition at elevated temperature High utility cost for elevated temperature High operating cost for acid consumption
Alkaline	18 % NaOH T = 100 ℃ Time = 1h	30	High reaction rate	Low sugar yield Sugar decomposition by alkali attack
Hot Compressed Water (HCW)	T = 150 -250 ℃ P = 10-25 MPa Time = 20 Min	< 40	No environmental and corrosion problems Low maintenance Cost Relatively high reaction rate	Relatively low sugar yield

Table 4 : Relative monosaccharide composition (% w/ w) of residues and the solubilized fraction after microwave irradiation and pretreated only with water

Residue

Temp °C	Arabinose	Galactose	Glucose	Xylose	Mannose
Room temp	21.2 ± 0.4	5.7 ± 0.1	40.0 ± 0.4	30.5 ± 0.1	2.8 ± 0.1
140	18.0 ± 1.0	6.8 ± 0.6	35.7 ± 0.6	37.2 ± 0.6	2.4 ± 0.4
160	13.3 ± 0.4	4.2 ± 0.3	38.0 ± 0.3	38.7 ± 0.1	2.1 ± 0.1
180	5.6 ± 0.2	2.3 ± 0.1	72.4 ± 0.9	19.4 ± 0.1	1.9 ± 0.2
200	0.7 ± 0.1	0.9 ± 0.4	89.4 ± 0.9	7.3 ± 0.1	1.7 ± 0.2
220	0.5 ± 0.1	0.6 ± 0.4	95.7 ± 1.1	2.3 ± 0.4	1.0 ± 0.4
Optimum point	1.8 ± 0.6	2.0 ± 1.1	83.3 ± 3.4	10.5 ± 0.7	2.6 ± 1.0

Solubilized fraction

Temp °C	Arabinose	Galactose	Glucose	Xylose	Mannose
Room temp	33.4 ± 1.4	7.0 ± 0.5	46.8 ± 0.5	12.9 ± 1.3	2.6 ± 0.1
140	30.2 ±0.2	6.0 ± 0.4	8.8 ± 0.4	20.2 ± 0.2	2.3 ± 0.2
160	25 ± 0.1	5.3 ± 0.2	24.9 ± 0.1	27.1 ± 0.1	2.0 ± 0.1
180	22.5 ± 0.4	4.4 ± 0.1	39.0 ±0.8	42.5 ± 0.3	1.8 ± 0.3
200	20.3 ± 0.1	3.7 ± 0.3	42.6 ± 0.4	38.3 ± 0.1	1.4 ± 0.2
220	19.1 ± 0.3	3.0 ± 0.4	38.6 ±0.1	33.2 ± 0.3	1.0 ± 0.1
Optimum point	22.5 ± 0.4	7.2 ± 0.3	26.5 ± 0.1	41.6 ± 0.2	2.2 ± 0.2

Table 5: Independent variables of the FFD and the carbohydrates yield

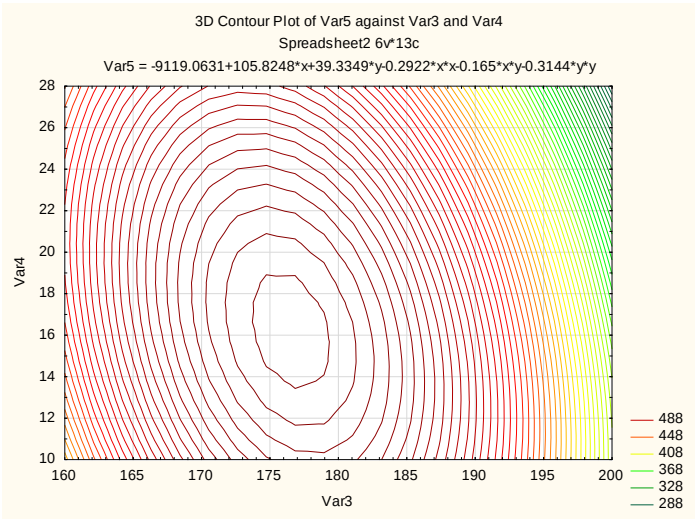
Run	Coded variables				Real variables				Carbohydrate yield (y)
					X_1	X_2	X_3	X_4	
1	0	0	0	0	150	2	0.75	3	92
2	0	0	0	0	150	2	0.75	3	87
3	0	0	0	0	150	2	0.75	3	84
4	-	-	+	-	140	1	1	2	42
5	+	-	+	+	160	1	1	4	119
6	-	-	-	+	140	1	0.5	4	49
7	+	-	-	-	160	1	0.5	2	95
8	-	+	+	+	140	3	1	4	72
9	-	+	-	-	140	3	0.5	2	70
10	+	+	-	+	160	3	0.5	4	161
11	+	+	+	-	160	3	1	2	186

Table 6: Regression analysis table

MODEL					
		Response#1			
Factor	Name	Coeff	P(2 Tail)	Tol	Active
Const		96.091	0.0000		
X_1	X_1	41.000	0.0004	1	X
X_2	X_2	23.000	0.0067	1	X
X_3	X_3	5.500	0.3695	1	X
X_4	X_4	1.00000	0.8658	1	X
	R^2	0.9208			
	Adj R^2	0.8679			
	Std Error	16.0359			
	F	17.4314			
	Sig F	0.0019			
	F_{LOF}	23.1160			
	Sig F_{LOF}	0.0419			
	Source	SS	Df	MS	
	Regression	17930.0	4	4482.5	
	Error	1542.9	6	257.2	
	$Error_{Pure}$	32.7	2	16.3	
	$Error_{LOF}$	1510.2	4	377.6	
	Total	19472.9	10		

Table 7: Independent variables, experimental and predicted carbohydrate yields of the CCD

	Coded variables		Real variables		Carbohydrate yield (mg / g)	
Run			X1	X2	Experimental	Predicted
13	-1.68	0	165	19	507	503
14	-	-	170	14	522	522
15	-	+	170	24	506	516
16	0	-1.68	180	12	530	532
17	0	0	180	19	542	532
18	0	0	180	19	530	532
19	0	0	180	19	514	532
20	0	0	180	19	535	532
21	0	0	180	19	534	532
22	0	1.68	180	26	511	499
23	+	-	190	14	488	488
24	+	+	190	24	439	449
25	1.68	0	195	19	432	427



Var 3 : X_1, Var 4 : X_2 , Var 5 : Y

Fig. 5 contour plot for central composite design

Conclusion

In this study, detailed effects of microwave irradiation were investigated for solubilization of carbohydrates from corn pericarp. Xylan could be separated from cellulose by MAE. The optimum condition for MAE of carbohydrates was determined by using RSM including fractional factorial design and central composite design. The optimized condition was as follows; heating temperature 176.5 °C, come-up time 2 min, heating time 16 min and solid to liquid ratio 1/20 (g/mL), respectively. Moreover, the optimum condition was also desirable for production of xylo-oligosaccharides. Surface plot and contour plot was drawn for fractional factorial design and central composite design respectively.

Acknowledgement:

We are very grateful to Coimbatore Institute of Technology, Coimbatore for providing facilities to carry out review work by access through various international journals.

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