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Analysis and Optimization of Solvent Free Microwave Assisted Extraction of Bio-oil from Orange peels using Response Surface Methodology

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Abstract - In present investigation, extraction of bio-oil from orange peels was examined for variable parameters like temperature and time using microwave assisted extraction technique without solvent usage. The extraction parameters were optimized using Central Composite Rotatable Design (CCRD) of response surface methodological approach to obtain high bio-oil yield. The physio-chemical properties of the extracted oil was determined according to standard methods, while the chemical composition was obtained using gas chromatography coupled to mass spectrometry (GC-MS). A quadratic regression model developed and diagnosed to estimate the optimum conditions for bio-oil recovery was validated in terms of Desirability (D) function. The optimal conditions of extraction established with CCRD, response surface methodology were extraction temperature, 113 ºC and time, 32 min. At these optimized conditions the predicted yield and desirability was 3.9 % and 0.78 respectively. The validation of predicted optimized conditions were done by performing an experimental extraction run at same conditions and the actual experimental bio-oil yield obtained at these conditions was 3.5 % which is in good agreement with the predicted result.

Keywords - *Microwave extraction; orange peel; bio-oil; optimization; response surface methodology.*

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I. INTRODUCTION

The utilization of several bioactive matter of plant origin in different commercial sectors such as cosmetics, pharmaceutical, food and flavoring industries have gained the momentum in recent years [1]. India being second largest producer of fruits and vegetables is generating ample residual biomass from food processing industries. Among the high production fruits such as mango, banana and citrus fruits cultivated in India, about 50 % of citrus fruit are used for juice processing resulting into significant amount of waste, mostly in the form of peels. Oranges (mandarin), a most popular variety of citrus fruit, is cultivated on near about 1.35 lakh hectare area of Maharashtra region and has annual production of 3.92 lakh metric tons [2]. A considerably large amount of orange peels have been disposed as industrial waste every year, thus causing serious environmental problems, particularly water pollution due to the presence of several water solvable bio-materials [3,4]. Over the last two decades, the recovery of bio-materials from orange peels such as polyphenolic compounds including flavonoids and phenolic acids, pectin and essential bio-oil has become the area of research due to the potential applications of the isolated bio-materials in cosmetics, flavoring, pharmaceutical and agro-industry [5-8]. In particular, the bio-oil extracted from orange peels is used to add aroma to the products such as carbonated beverages, air fresheners, perfumes etc [9]. Further, bio-oil contains δ-limonene as major constituent which has chemo preventive activity for skin, liver, lung and stomach cancer [10]. Moreover, the bio-oil possesses some germicidal, antimicrobial and antioxidant properties contributed from inherent polyphenolic compounds [11].

Extraction is one of the key technologies for sustainable growth and economy of agro-food process industries. The use of various conventional extraction techniques such soxhlet extraction, maceration, hydrodistillation has been reported to obtain the high value commercial bio-oil [1,12,13]. However, these conventional methods are associated with limitations related to higher energy consumption, higher extraction time, environmental safety, materials safety and control issues [4,14]. Thus, for eliminating the environmental impacts, reducing the cost, shortening the extraction time and reduction of organic solvent consumption the novel green technologies need to be developed [5,6]. Among the newer techniques microwave assisted extraction (MAE), ultrasonic extraction (USE) and supercritical fluid extraction (SFE) are used as the green processes for enhancing the extraction performance [15-18]. The MAE method is based on direct application of electromagnetic radiation to organic solvent or plant tissue which having the ability to absorb electromagnetic energy (microwave radiations) which is transformed into heat [19]. The MAE leads to faster processing, improved yield and quality of product, direct extraction capability, lower energy consumption, reduced solvent levels, and lower capital investment compared to conventional extraction methods [20-23].

The main objective of this work was to develop microwave assisted extraction of bio-oil from orange peels without addition of any solvent and optimize the process conditions for maximum extraction yield of bio-oil using the Central Composite Rotatable Design (CCRD) of response surface methodology (RSM). RSM is a collection of statistical and mathematical techniques suited for solving nonlinear data processing issues [24,25]. In this study, RSM has offered advantages of relatively simple calculation, less number of experiments, short cycle and regression equation with high accuracy as compared to traditional laboratory experimentation method which is highly laborious, time consuming and less credible [25-26].

II. MATERIALS AND METHODS

Orange Peels

Fresh Mandarin orange fruits (*Citrus reticulata*) were obtained from local market of Jalgaon district, Maharashtra, India. The fruits were peeled by hand and separated from the non bio-oil matter, external fibrous *flavedo*. The yield of peels after peeling was expressed as percentage of initial weight of fresh orange fruits. The moisture content and total oil content of the dried peels was determined by using standard methods of FSSAI, 2012.

Experimental design

The method of designing of experiments (DoE) was selected to analyze the effects of two independent variables, temperature and time on the predicted response, bio-oil yield [27]. A central composite rotatable design (CCRD) using the software Design Expert 9.0.1 was applied to evaluate the model for prediction of bio-oil yield with intention to reduce the number of extensive experiments and to arrange the experiments with several interactive combinations of independent variables. The experimental ranges and levels of the factors used in the CCRD design for microwave assisted solvent free extraction (MASFE) of peels are given in Table 1. The experimental design matrix of the test factors were coded according to the following equation:

$$x_i = X_i - Xo/\delta X_i \qquad (1)$$

where, x_i is the dimensionless coded value of the i^{th} independent variable; X_i the natural value of the i^{th} independent variable at coded level; X_o the natural value of the i^{th} independent variable at the center point and δX_i the step change value.

Once the experiments were performed, the experimental results were fitted with 2nd order polynomial equation.

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{11}x^2_1 + b_{22}x^2_2 + b_{12}x_1x_2 + e \qquad (2)$$

where, Y is the predicted response; b_0 the intercept; b_1, b_2 the linear co-efficient; b_{11}, b_{22} squared co-efficient and b_{12} the interaction coefficients and e is random error.

Table 1: Experimental ranges and levels of Independence variables

Independent Variables	Coded Variables				
	-α	**-1**	**0**	**+1**	**+α**
A Time (min)	15	30	45	60	75
B Temperature (^oC)	90	100	110	120	130

Microwave assisted solvent free extraction

Solvent free microwave assisted extraction of bio-oil from orange peel was carried out in a MAS-II (Sineo) microwave apparatus. The multimode microwave reactor is equipped with twin magnetron (2 × 800 W, 2450 MHz) with a maximum delivered power of 1000 W variable in 10 W increments. A rotating microwave diffuser ensures homogeneous microwave distribution throughout the plasma coated PTFE cavity (35 cm × 35 cm × 35 cm). The temperature was monitored by a shielded thermocouple (ATC-300) inserted directly into the corresponding container. In a typical MASFE procedure, the known weight of fresh orange peels were subjected to extraction by application of microwave power of 300 W without addition of any solvent or water. In the process, the temperature of extraction was varied from 90 to 130 °C for 15-75 minutes to release the bio-oil from peel through *in-situ* evaporation of fatty matter along with internally bound water. The bio-oil yields at different operating conditions were noted and were stored to below ambient temperature.

Analysis of bio-oil

Several important physical and chemical properties of the recovered bio-oil such as color, refractive index, optical rotation, specific gravity, solubility, free fatty acid content, saponification value, iodine value and peroxide value was determined according to the standard method of FSSAI, 2012 [28].

The chemical composition of bio-oil was determined by gas chromatography (GC) coupled with a mass spectrometer (MS) using a THERMO GC (TRACE 1300) with a fused silica capillary column, PE-5 (50m× 0.32mm, film thickness 0.25µm) and a triple quadrupole Thermo MS (TSQ 8000) mass spectrometer. A sample of 1.0 µL was injected in the split mode with split ratio 100:1. An electron ionization (EI) system, with electron energy of 70 eV and emission current 200 µA was used for GC-MS detection. Helium was used as a carrier gas at a flow rate of 1.1 mL/min and ionization temperature was kept at 250 ^oC. The GC-MS was equipped with Dyna Max XR detection system having discrete dynode electron multiplier and electrometer. The mass scanning range was varied over low mass (m/z), 40 to high mass (m/z), 450 and acquired total scan 8844 with mass resolution of 1.5 µ for run time of 30.08 min. The components of the bio-oil were identified by their retention time and compared with mass spectrum data from the National Institute Standard and Technology (NIST) library available with the GC-MS system. The relative amounts of individual compounds were calculated based on the GC peak areas.

III. RESULTS AND DISCUSSION

The peel content of fresh orange fruits was found to be 220 gm/kg. The moisture and total oil content of the peels on wet basis was found to be 79.2 % and 12.9 % respectively.

Physico-chemical Characterization

The result of physico-chemical properties determined for bio-oil from orange peels in present work is shown in Table 2. These results are in agreement with the findings of *Khan et al., 2013* [10] and *Javed et al., 2014* [29].

Chemical Composition by GC-MS Analysis

GC-MS chromatogram of bio-oil obtained from orange peels using solvent free microwave assisted extraction is shown in Fig. 1, where the two main peaks are located at retention time of 5.09 and 5.75 min, which are identified as limonene and decanal. In total 8 major peaks were recorded and correlated to the components of the bio-oil by identification methodology. The chemical composition of orange peel bio-oil is listed in Table 3. As far as the groups of chemical constituents are concerned, the bio-oil is found to mainly consist of monoterpene hydrocarbons and oxygenated monoterpene hydrocarbons. In addition, some monosaccharide and disaccharide sugars along with phenone and ester groups were derived from peels suggesting the possible higher content of pectin.

Table 2: Physico-chemical properties of bio-oil from orange peels

Properties	
Color (Lovibond cell 5 ¼″)	15Y
Refractive index (nD^{25})	1.4337
Optical rotation (25 ^oC)	+88
Specific gravity (25 ^oC)	0.915
Saponification value (mg KOH/g)	146.6
Iodine value (g I_2/100g)	97.8
Acid value (mg KOH/g)	5.4
Peroxide value (mg eq/kg)	37.2
Solubility	
Water	Insoluble
Hexane	Soluble
Acetone	Fairly soluble

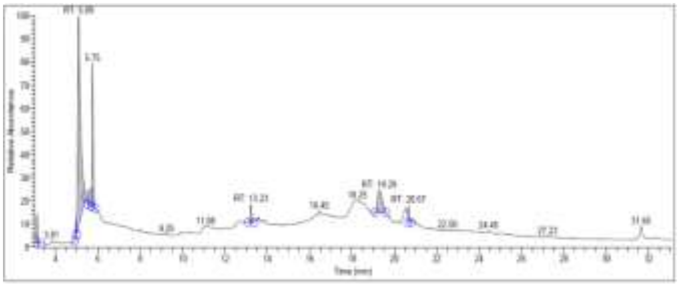

Figure 1. GC–MS spectrum of the bio-oil obtained from orange peels using MASFE extraction technique.

Table 3: Chemical composition of bio-oil from orange peels identified by GC–MS

Peak	Retention time (min)	Identified compound	% Area
1	3.13	*α*-pinene	3.03
2	4.96	*β*-myrecene	1.56
3	5.09	Limonene	61.69
4	5.67	*α*-terpineol	4.48
5	5.75	Decanal	13.97
6	13.23	4-Hydroxy-2-methylacetophenone	2.85
7	19.29	*β*-D-Glucopyranose, 4-O-*β*-D-galactopyranosyl	10.51
8	20.67	Phthalic acid, butyl hept-4-yl ester	1.90

Experimental design and model fitting

In present study, the said domain of two independent variables (temperature and time) was feed in software using CCRD which resulted in thirteen experimental designing runs. These runs were performed to estimate six coefficients of the second order polynomial equation and subsequently used to predict the response, yield. The experimental design matrix derived from CCRD and the results of each experimental extraction run for oil yield are shown in Table 4.

The minimum yield (1.48 %) was observed in run number 7 (time central level and temperature at negative α), while the maximum yield (5.22 %) was recorded in run number 2 (time at +1 level and temperature at -1 level). The coefficient of the model for the response was estimated using multiple regression analysis technique included in the RSM. The variables analyzed for their quadratic effects resulted in Eq.(3) (in terms of coded unit) to predict the oil yield within the experimental domain.

$$R_1=4.66+0.44A+0.066B-0.32AB-0.57A^2-0.56B^2 \quad (3)$$

The generalized final quadratic model in terms of actual factors is given in Eq. (4).

$$Yield = -81.50+0.49(Time)+1.34(Temp.)-2.16(Time\times Temp.) -2.53(Time)^2-5.64\,(Temperature)^2 \quad (4)$$

The oil yield, measured in terms of percent extraction was significantly affected by both the independence variables at their quadratic level. The positive sign in front of term indicate synergetic effect were as negative sign indicates antagonistic effect [30].

Table 4: Experimental design matrix for two independence variables and results for oil yield

Std. order	Run order	Factor 1 A:Time (min)	Factor 2 B:Temperature (°C)	Response Bio-oil Yield
1	1	30	100	3.48
2	2	60	100	5.22
3	3	30	120	2.88
4	4	60	120	3.32
5	5	15	110	1.50
6	6	75	110	3.05
7	7	45	90	1.48
8	8	45	130	3.12
9	9	45	110	4.58
10	10	45	110	4.58
11	11	45	110	4.58
12	12	45	110	4.58
13	13	45	110	4.58

Analysis of variance (ANOVA) evaluation

In the present work the adequacy of the model was justified through analysis of variance (ANOVA). The results of the quadratic model for percent yield in the form of ANOVA are given in Table 5. The actual and the predicted bio-oil yield is shown in Fig. 2. Actual values are the measured response data for a particular run, and the predicted values are evaluated from the model using the approximating functions. In Fig. 2 the values of R^2 (0.82) and adjusted R^2 (0.69) is approaching to unity and thus the model can be considered suitable to define linear relationship between observed value and predicted value. The acceptability of model was verified by employing Fisher's test. In general, the Fischer's '$F_{statistics}$' value with a low probability 'P' value indicates high significance of the regression model. The values of "$P > F_{statistics}$" for the model less than 0.0500 indicates that the model terms are significant, while the terms with values greater than 0.1000 shows non-significance in empirical model. In present extraction methodology, the model terms A^2 and B^2 are significant model terms, while the other terms has less influence on percent yield. Besides, the model F-value of 6.38 implies that the model is significant for prediction of response, yield and thus, signifies the quadratic relationship between the independent variables and response.

Table 5: Response surface quadratic model analysis of variance (ANOVA) table

Source	Sum of Squares	Df	Mean Square	F Value	p-value Probe > F
Model	14.29	5	2.86	6.38	0.0153
A	2.33	1	2.33	5.19	0.0568
B	0.052	1	0.052	0.12	0.7431
AB	0.42	1	0.42	0.94	0.3643
A^2	7.46	1	7.46	16.64	0.0047
B^2	7.29	1	7.29	16.27	0.0050
Residual	3.14	7	0.45		
Lack of Fit	3.14	3	1.05		
Pure Error	0.000	4	0.000		
Cor Total	17.43	12			

Interaction effects of extraction parameters

The interaction effect of the process variables, time and temperature on the percent yield was investigated using response surface methodology. The quadratic regression model was used to represent interactions results graphically in two-dimensional (2D) contour and three-dimensional (3D) response surfaces plots (Fig. 3 and 4). The maximum to minimum values of response based on the interaction of the two variables were represented by the color of the 3D surface and contour which is red to green, respectively. These plots shows that the oil yield improved with increase in time but temperature rise do not significantly affect the yield. For instant from Fig. 3, at time, 30 min and temperature, 120 ºC the oil yield was 2.88 % which increased to 5.22 % with time, 60 min and temperature, 100 ºC. The maximum yield was observed at boiling temperature of internal moisture.

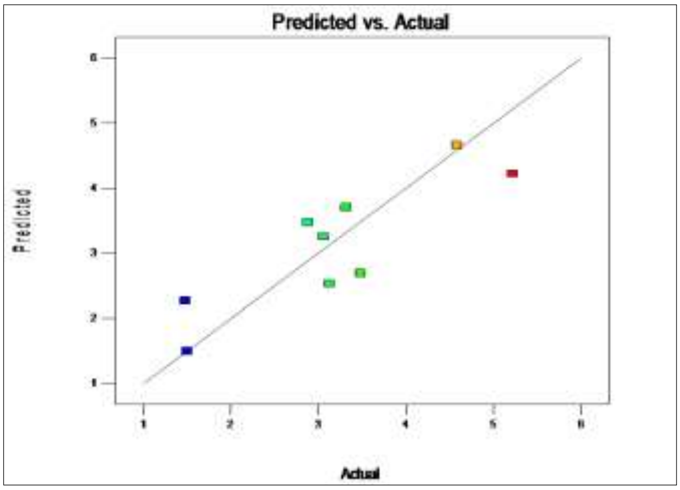

Figure 2. The actual and predicted plot of bio-oil yield

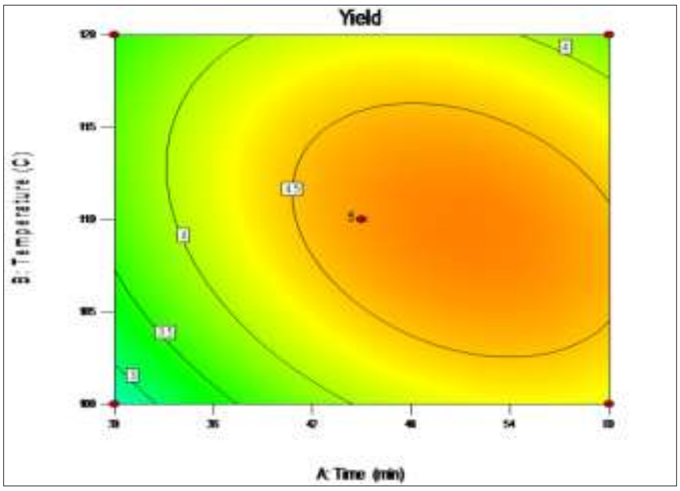

Figure 3: Counter plot showing effect of time and temperature on percentage of oil yield

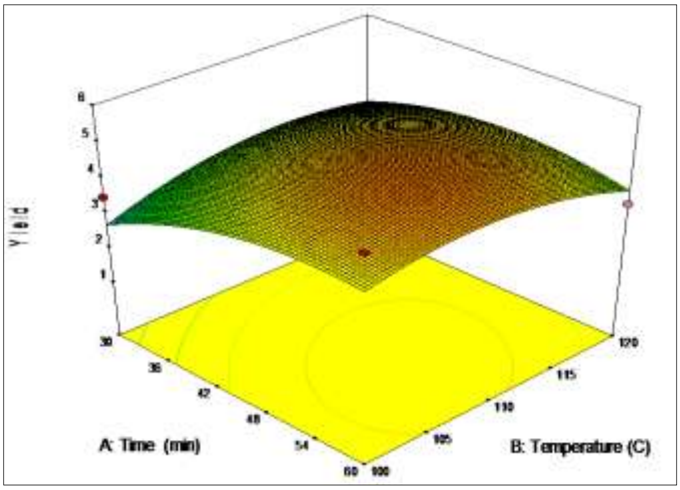

Figure 4: Three dimensional plot showing effect of time and temperature on percentage of oil yield

Optimization and validation by response surface modeling

The optimization of the process parameters as an individual or combined was done to achieve best response, yield. The quadratic model equation was optimized with target to maximize the yield and minimize the time of extraction, keeping the temperature within experimental range of 100-120 ºC. This objective was described in term of desirability function which combines the overall effect of variables. Figure 5 represents the three dimensional graph of optimized desirability where it indicates the highest yield up sight of the graph. The solution generated for optimal conditions of extraction established with CCRD for specified limits were extraction temperature, 113 ºC and time, 32 min. At these optimized conditions the predicted yield and highest desirability was observed to be 3.9 % and 0.78 respectively.

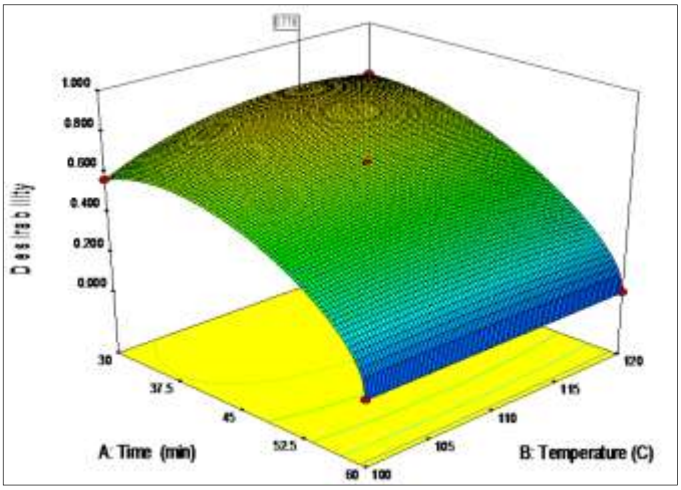

Figure 5: Desirability plot time with temperature

The validation of the predicted optimized conditions were done by performing an experimental extraction run with the parameters suggested by model and the actual experimental bio-oil yield obtained at these conditions was found to be 3.5 %. The actual experimental value is in good agreement with the predicted one which shows that the developed quadratic regression model of CCRD stood valid for the given sets of parameters and could be successfully applied for predicting the extraction yield without further experimental runs.

IV. CONCLUSION

In this research, the sustainable recovery of the bio-oil from orange peel waste was achieved employing no solvent for extraction. Further, the application of MAE technique proved to be an effective way to shorten the extraction time and able to extract the bio-oil consisting limonene and decanal as the major chemical components. MAE was optimized for obtaining high yield of bio-oil as response variable, where two factors central composite response surface experimental design was successfully employed to determine the interactive effect of temperature and extraction time. The optimized conditions established for MAE in bio-oil extraction were temperature, 113 ºC and time, 32 min with maximum bio-oil yield 3.9 %. Under optimal conditions, the experimental value of bio-oil yield, 3.5 % agreed satisfactory with the predicted value, 3.9 %. These result demonstrated that the CCRD model developed for MAE of bio-oil from orange peels can be successfully utilized to predict the extraction yield at varying operational conditions without further experimental runs.

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