Supercritical Carbon Dioxide Extraction of Clove (Syzygium Aromaticum) Bud Oil in an Annular Grate Feed Contactor: Effect of Axial To Radial Surface Enhancement Factor

Sutapa Roy, Chandan Guha, Asit Kumar Saha, Somak Jyoti Sahu

Abstract: ***Experimental investigations were carried out with and without incorporating a perforated concentric tube (diameter 0.75 cm or 0.15 cm with one blind end at the upstream side) within a typical cylindrical extraction bed. A new parameter, axial to radial surface enhancement factor, $[(r_o-r_i)/2L]$, was defined to correlate the effect of bed geometry with extraction performance. Supercritical CO_2 extraction of clove bud for essential oils was carried out with three different bed geometry at three different pressures (14.7, 19.6, & 24.5 MPa) and temperatures (35^0C, 40^0C & 45^0C) combination. Detailed analysis of the extraction rate data was presented and found to have two distinct rate periods, namely constant extraction rate period and falling extraction rate period with a small transition period in-between. Effects of bed geometry, temperature, and pressure on the oil yield were analyzed statistically using face centered central composite design technique. The optimum conditions were identified as 24.5MPa pressure, 44.72 °C temperature and bed type with 0.15cm perforated concentric tube with the optimal yield of 17.981.***

Keywords: Supercritical carbon dioxide extraction, Clove oil, central composite design, kinetic study, Surface enhancement factor.

I. INTRODUCTION

The ever increasing demand of essential oils is due to their scented biodegradable aromatic compounds used in various applications as flavouring agents in food, beverages, confectionery; pharmaceutical aids; aroma chemicals in aromatherapy; perfumery and cosmetic ingredients; "green pesticides" and insect repellents [1-11]. Worldwide the commercial interests for essential oil production, isolation of its components of potential interest are gradually growing along with searching more and more species enriched with bioactive green components and cultivating them collecting from their wild origin for expansion of world market of natural essential oils. India is one of the world's largest producer, consumer and exporter of essential oils [12, 13]. The favourable climatic conditions and quality soil suitable for the agricultural growth of aromatic plants, continuous development in science and technology and huge investment in processing and trading essential oils make India to play the dominant role in the world market of aroma producing plants and essential oils.

Over the years, extensive research works are going on for continuous growth of the Indian essential oils based economy by developing new agro and extraction technology-based, quality-monitoring, customer-centric, market-driven industry. Since quality of the product has been given great importance in recent times, supercritical fluid extraction (SFE) technology using CO_2 as a solvent is established as an efficient and effective novel technology for obtaining valuable bioactive compounds in more concentrated form than other traditional methods for various plant extracts [14-20]. The extract obtained by supercritical carbon dioxide extraction (SCO_2E) is free of any solvent impurity which is the most attracting factor for this method becoming popular overall traditional methods facing severe problems by environmental regulatory acts due to the use of organic, hazardous, toxic solvents in their methods [21].

SCO_2E system consists of an extraction unit, extractor, which is the heart of the system, is a high-pressure vessel where actual extraction operation of natural biomass is carried out. Geometrical parameters of extractor have a significant influence on the overall extraction curve (OEC). Most of the research work in this field have been focused on mass transfer rate; phase equlibria; effect of various parameters such as temperature, pressure, particle size, solvent flow rate, maturity of biomass; characterisation of the extracts; and cost of extraction [22-26]. Effect of bed geometry on extractor performance in terms of bed volume, bed height to diameter ratio and related cost analysis were reported in some literature [27-31]. Still, bed geometry is gaining interest of researchers for enormous studies on the impact of bed geometry on extraction kinetics and scale up of SFE processes.

In this research work, a unique concept is introduced in the extractor bed geometry modifying the conventional pattern and effects of temperature and pressure on extraction of clove bud essential oil in this modified bed geometry were studied.

Revised Manuscript Received on April 06, 2019.

Sutapa Roy, Department of Chemical Engineering, Haldia Institute of Technology, Haldia 721657, West Bengal, India

Chandan Guha, Department of Chemical Engineering, Jadavpur University, Kolkata, India

Asit Kumar Saha, Department of Chemical Engineering, Haldia Institute of Technology, Haldia 721657, West Bengal, India

Somak Jyoti Sahu, Department of Chemical Engineering, Haldia Institute of Technology, Haldia 721657, West Bengal, India

At the same time effects of bed geometry on extraction kinetics were studied comparing the extraction curves. Clove is chosen as raw material considering various factors. (i) Among 10% essential oil bearing plants from giant plant kingdom most contain very low quantity barely exceeding 1% essential oil [32]. High essential oil content (more than 10%) is found in very few plants species. Clove (*Syzygium aromaticum*) and nutmeg (*Myristica fragrans*) are common examples in this category. Reports say that aromatic oil content of clove buds varies in the range of 15-20% [33]. (ii) Clove bud essential oil is ease to separate from its matrices applying moderate pressure and temperature (reported 40^0C and 15MPa by Prado et. al., 2011[34]) by SCO_2E compared to many more plant materials (suffering from high-temperature, high-pressure operations) and better quality product is possible to obtain using this technology [22]. (iii) Clove is champion among all spices. It has wide range of applications in food, cosmetic, and pharmaceutical industries for its major bioactive components eugenol, β-caryophyllene and α-humulene etc. as antioxidant, antimicrobial, antibacterial, anti-inflammatory, antiviral, antifungal, anti-diabetic, anti-carcinogenic, anesthetic, antiseptic, analgesic, etc. [33, 35-37]. Thus, selection of clove buds as "model raw material" is ideal for SCO_2E studies.

II. MATERIALS AND METHODS

A. Raw Materials and Chemicals

The quality flower buds of clove as available in local market were purchased from Haldia (West Bengal, India), checked thoroughly for removal of foreign materials (if any) and dried at 30°C in a laboratory scale air circulated drying unit for 12 h, in order to control the moisture content of the feed mass below 12% to avoid the impact of moisture on the volatile matter, mass transfer rate and solubility of the extract in the solvent CO_2 [38, 39].

The air dried clove sample was then ground into smaller particles in a mixture grinder (Philips Mixer Grinder HL7720) and stored in a cold place in air-tight containers for subsequent experimental studies on SCO_2E. The ground material was classified with sieves of mesh sizes 16 -42 from Tyler standard screen series. The particle size (D_P) of 0.64mm was obtained following the mass mean diameter calculation.

CO_2 (Commercial grade with above 99% purity) was purchased from Bharat Oxytech Pvt. Ltd., Haldia (West Bengal, India) and used as solvent for the SFE.

B. Moisture Content of Clove Buds

The moisture content of ground material was determined using the "SARTORIUS MA45C" moisture analyzer before and after drying. The moisture analysis results were prepared after triplicate measurements.

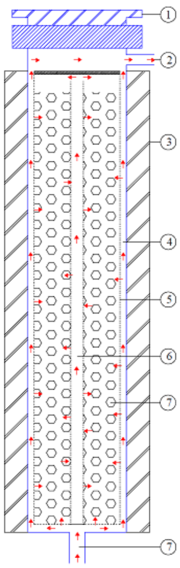
C. Determination of the Total Amount of Extractable Material (Global Yield)

The total extractable essential oil content from clove buds was determined using conventional solvent extraction method. To perform the experiment 30 gm dried ground clove bud sample from the same feedstock, as used for SCO_2E studies, was wrapped in a filter paper and loaded in a glass thimble which was connected with 500 mL capacity reflux flux connected with Soxhlet apparatus. Extraction was carried out using 300 mL n-hexane for 6hrs. The clove oil extract was concentrated by removal of solvent at 50^0C in a rotary vacuum evaporator.

D. Experimental Set-up and SFE Process

Extraction operations of clove buds essential oil were experimentally performed using a semi batch flow type SFE module (Model No: CSL/SCF/1L2/400) purchased from M/s Chemtron Science Laboratories Pvt. Ltd., Navi Mumbai, India. It is equipped with one high-pressure pump for solvent delivery (HP DOSING *PUMP* MODEL# *UMBL-30:* PLUNGER), one supercritical CO_2 generator, two parallel connected 1L Extraction vessels (each of 42cm height and 5.5cm inside diameter with maximum operable pressure of 29.42Mpa), and two low pressure Separators (each of 1L capacity) connected in series, and a refrigeration unit (design lowest temperature -14^0C). The extraction vessels, supercritical fluid generator and separators all are covered with heating jackets to keep temperature constant to the set point.

The solid feed samples were fed inside the extractor with the help of an externally loaded cylindrical shell having perforated surface. In this study a special modification in bed geometry was introduced by inserting a perforated small diameter concentric tube (with one blind end at the upstream side) inside this external cylindrical shell and ground clove buds was charged in the annular space of the modified bed. Two different diameters (0.75cm and 1.5cm) were chosen to design the annular bed geometry. The influence of annular solid beds (B2, designed using 0.75cm diameter inside channel & B3, designed using 1.5cm diameter inside channel) and conventional cylindrical solid bed without any internal annular path (B1) were studied under varying operating conditions. The diagram of an annular solid feed bed loaded inside the extractor is shown in Fig.1.



1. Extractor cap
2. Solute (essential oil) rich supercritical carbon dioxide
3. Thermostatic water bath
4. Extractor vessel
5. Perforated annular feed bed
6. Perforated concentric tube
7. Ground botanical mass
8. Supercritical carbon dioxide

Fig. 1: Supercritical Fluid Extractor Bed Design

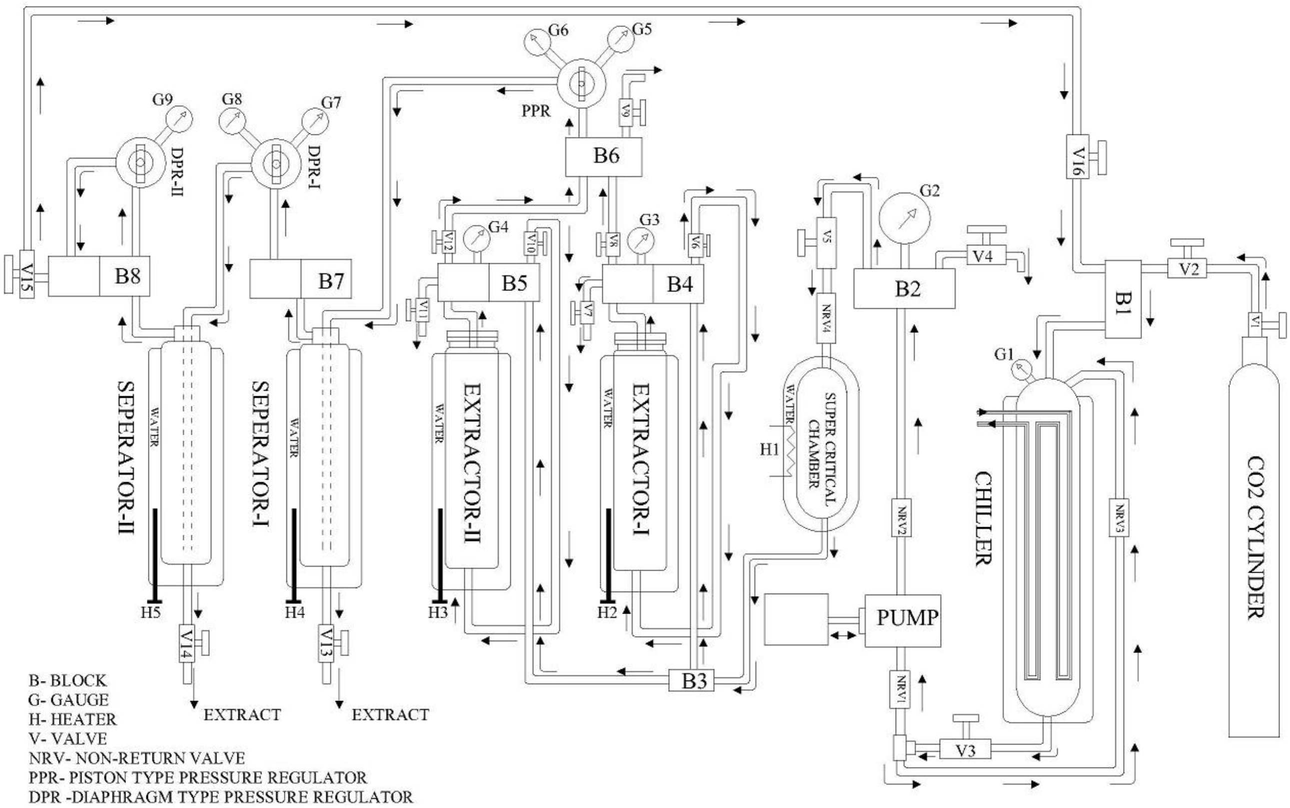


Fig. 2: Schematic of Supercritical Fluid Extraction Setup

For experiment initially a particular type of extractor bed among above mentioned three types were chosen and packed with crushed clove powder while both ends of the bed were covered with polypropylene wool to prevent any solid loss and protect the extractor lines from blockage. This externally filled solid bed of feed was then loaded inside the extractor vessel to carry out extraction.

Schematic of SFE module used in the present study is shown in Fig. 2**.** (i) Solvent CO_2 is supplied from cylinder in liquid form (ii) and allowed to enter in a reservoir (≈6.37MPa) where its temperature reduces below 5^0C. (iii) Cold liquid CO_2 is then pressurized to desired operating pressure with the help of a high-pressure pump (maximum pressure limit 29.42 MPa). High-pressure pump head temperature is controlled with the help of a cold water circulation system through which cold water of 4^0C was circulated. (iv) Pressurized liquid CO_2 is heated up to attain the desired supercritical temperature (which is above critical temperature 31.1^0C) of extraction in the SCO_2 generator with the help of a thermostatic water bath. (v) Now it enters into the already charged extraction vessel where desired supercritical condition is maintained throughout the period of extraction. After introducing the solvent in the extractor, the outlet valve of the extractor is kept closed to provide a static extraction period (t_s) of 20 minutes before starting the dynamic extraction to ensure better contact between fluid-solid phases in all the experiments performed. In the extractor SCO_2 penetrates in the molecules of charged material (crushed Clove in this study) and extracts their essence. Desired flow rate of SCO_2 is maintained for 210 min (t_t) for all experiments. (vi) From extractor this extract rich solvent is allowed to pass through two successive steps of extract recovery vessels termed as Separator-I and separator-II. (vii) At extract recovery vessels, temperature and pressure are reduced in two steps to that extent (below 31.1^0C, and 7.4 MPa pressure) so that SCO_2 converted back into gaseous CO_2 using Manual Back Pressure Regulator. Thereby, solubility of extracted oil in the solvent CO_2 drops significantly and oil is separated and drained out from bottom of the separator vessels. In all experiments, the oil sample was collected and weighed at intervals using different sampling bottles and recorded to prepare OEC. (viii) After second stage of separation oil free CO_2 gas from the top of the separator-2 is re-circulated back to refrigeration section for reuse. (ix) After completion of extraction closing the inlet and outlet valves of extractor, it is vented to unload the oil extracted solid mass. (x) The total extract is centrifuged and separated from other co-extracts and kept under refrigeration until the analyses were carried out.

E. Experimental Design for Extraction

The extraction yield of essential oil and the presence of important bioactive ingredients in concentrated form in the extracted mass are influenced by various operating parameters, directly or indirectly, such as temperature, pressure, particle size, solvent flow rate, time of extraction, use of co-solvent, level of moisture in the feed, porosity of feed bed, extractor bed geometry etc. [40] and their effects on oil yield may be either independent or interactive [41]. In the present work modified extractor bed geometry was chosen as one important factor to study its effect on efficiently extracting clove oil.

To express annular bed geometry of feed material, a new dimensionless group, axial to radial surface enhancement factor (ARSEF) which is the ratio of axial surface [i.e., cross sectional area $\pi(r_o^2-r_i^2)$] to radial surface area [i.e., $2\pi L(r_o+r_i)$], is introduced as $((r_o - r_i)/2L)$, where r_o is the radius of outer cylinder and r_i is the radius of inner cylinder, and L is the length of the bed. Three parameters (i) bed geometry $X_1 = \frac{r_o - r_i}{2L}$, (ii) temperature (X2) and (iii) pressure (X3) were considered to study their effects after implementing this modified pattern of bed on percentage oil yield [%OY = (gm of oil extracted / 100 gm of feed)]. All the experiments were designed to conduct keeping other parameters such as particle size(D_P), mass of feed loaded (F), solvent flow rate (Q_{CO2}) and extraction time (t_E) constant at 0.64 mm, 600 gm, 18.5 gm/min and 210 minutes, respectively. The amounts of clove buds used in different runs were taken from same sample prepared initially and stored properly.

Table I: Three levels of selected variables chosen for FC-CCD under RSM

ARSEF, $X_1 = \frac{r_o - r_i}{2L}$	Temperature, X2 (^{0}C)	Pressure, X3 (MPa)
0.0238 (-1)	35 (-1)	14.7 (-1)
0.0283 (0)	40 (0)	19.6 (0)
0.0327 (+1)	45 (+1)	24.5 (+1)

In this investigation, a statistical model was proposed to optimize the above mentioned three parameters for SCO_2E of clove buds oil. The statistical optimization procedures had huge applications in this field since it considers various possible interactions of variables during optimization [42]. Face centered central composite design (FC-CCD) was applied as an experimental design strategy to study the influence of these three factors on the %OY as response. In statistical design and analysis, central composite designs (CCD) are experimental designs under Response Surface Methodology (RSM), a powerful tool for process optimization [41, 43-45, 26]. Three independent variables (X1, X2, and X3) were tabulated at three levels which are generally coded as (−1), (0) and (+1). Twenty different combinations of these three independent variables are generated in face centered FC-CCD. The temperature and pressure conditions selected were 35^{0}C (-1), 40^{0}C (0), and 45°C (+1) and 14.7 MPa (-1), 19.6 MPa (0), and 24.5 MPa (+1), respectively. The minimum temperature (35°C) was chosen considering the critical temperature of the CO_2 (31.1°C) and the maximum (45°C) was set following the optimum temperature (40^{0}C) reported in earlier works [34] as well as to avoid extraction of unwanted compounds and possible thermal degradation of the extract at high temperature. Regarding the operating pressure, the minimum pressure of 150 bar was chosen to obtain a high solvation power related proportionally with density of CO_2 at all levels [46, 47] and a maximum value of (250 bar) was selected according to the study of Mukhopadhyay and Rajeev, 1998 [48]. Three values of the dimensionless parameter X1 used in this study were 0.0238(-1) for annular bed (B3), 0.0283 for annular bed (B2) and 0.0327 for conventional cylindrical feed bed (B1). Three levels of each of these three variables are represented here by Table I. All the 20 set experiments based on CCD combinations were performed and responses (% OY) were recorded and the analysis of variance (ANOVA) of each independent factor was performed using Design Expert-11 statistical package [43]. Thus, the influence of each independent factor and their interactions were examined and estimated statistically.

F. Kinetics of Clove Oil Extraction in Annular Feed Bed Using SCO2E

Extractor feed bed geometry is an important factor in the design and scale-up of a SFE process. Success of scale up procedure depends on the quality data of kinetic study obtained from OECs at lab scale or pilot plant scale experimental set up. In this study three different feed beds (B1, B2 and B3 as mentioned in section D) with two types of bed geometries were used. Thus the evaluation and comparisons of OECs for all these three bed geometries are very much relevant for future scale up.

All the experiments to develop OECs were conducted for three different beds (B1, B2 and B3) for three different temperatures (T1=35^{0}C, T2=40^{0}C, and T3=45^{0}C) using the same experimental set up used in FC-CCD study. Other parameters such as mass of feed loaded (F), extraction pressure (P), Particle size(D_P), solvent flow (Q_{CO2}), initial static period of extraction (t_S) and extraction time (t_E) were kept constant for all runs. For each run, 15 samples of clove oil were collected initially at an interval of 10 minutes up to 90 minutes followed by 20 minutes interval up to 210 minutes. All the experiments were performed twice in similar conditions. The experimental data are provided in Table II. The yield was expressed as %OY as mentioned in section E. The OECs obtained from various experiments were adjusted with two straight lines and mentioned as constant extraction rate (CER) and falling extraction rate (FER) periods using graphical method [29]. Kinetic parameters like (i) constant extraction period (t_{CER}), (ii) the rate of extraction during CER (R_{CER}), (iii) % yield achieved during CER (Y_{CER}), and (iv) percentage of total yield (%OY) were presented adjusting the OECs to compare the curves obtained from various feed bed geometry [34].

Table II: Experimental data of kinetics studies

F (gm)	Q_{CO2} gm/min	T in E (C^0)	P in E (MPa)	T in S_1 (C^0)	P in S_1 (MPa)	T in S_2 (C^0)	P in S_2 (MPa)	t_s (min)	t_E (min)
600	18.5	35/40/45	19.6	33	≈6	28	≈5	20	210

F - Mass of feed, Q_{CO2} - Solvent flow, T – Temperature, P - Pressure, E – Extractor, S_1 – Separator1, S_2 – Separator2, t_s - Static period, t_E – Extraction time

G. Clove Oil Characterisation by GC/MS analysis

Qualitative and quantitative analysis of essential oil of clove buds was performed to identify the components present in it based on chromatographic with a flame ionization detector and spectroscopic criteria. In present work *GCMS*-QP2010 SE (SHIMADZU, Kyoto, Japan), an advanced standard gas chromatograph *mass* spectrometer, was used for this analysis. It was equipped with DB - 1 MS UI capillary column (length 60m, inside diameter 0.25m, internal film width 0.25µm) for separating the components supplied by Agilent. The essential oil sample of clove buds was diluted using acetone in 1:4 ratios (1% oil and 4% acetone) for chromatographic injection with the help of auto injector. 1 µL volume of sample was injected in the split mode (1:50). The carrier gas Helium (He) flowed maintaining the flow conditions of (i) total flow 53.3 mL/min, (ii) column flow 0.50 mL/min, (iii) purge flow 3.0 mL/min and (iv) pressure 63.3kPa. Column oven temperature was gradually increasing from 50^0C, maintained for 3min, and then increased gradually at the rate of 1^0C min^{-1} for 10 minutes, 2^0C min^{-1} for 40 minutes and 3^0C min^{-1} for 30 minutes until 230^0C, maintaining isothermal condition for last 7 minutes. The total run time was 90 min.

Mass spectroscopic detector settings used were – (i) Ion source temperature 220^0C, (ii) interface temperature 300^0C. The obtained mass spectra can be matched using GCMS solution software (version 4) developed with MS library - NIST, Wiley, and SHIM. All the testing of samples was done in quality control laboratory of M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India.

III. RESULTS AND DISCUSSIONS

A. Moisture content and Global Yield :

Moisture content of raw sample of clove buds purchased from market was 13.25%. Moisture content of feed sample used for the extraction after drying and grounding was 6.83%. The global yield obtained from Soxhlet extraction procedure was 19%.

B. Analysis of Variance (ANNOVA)

Based on the FC-CCD combinations for three process variables (X1, X2, and X3) of SCO_2E of clove buds essential oils, the results of all runs (i.e. responses of FC-CCD) in terms of %OY and corresponding values of independent factors were reported in Table III. For fitting suitable model, all experimental data were analysed for linear, two-factor interaction (2FI) and quadratic models for the responses %OY based on R^2 [49], standard deviation, adjusted R^2, predicted R^2, “PRESS” value F-values, p-values and lack-of-fit tests results. The quadratic model presented highest F-value (205.23) and p-value < 0.0001 i.e. the quadratic model showed insignificant lack of fit for data. This model came out as best for exhibiting low value of standard deviation (0.0854), high value of R^2 (0.9978) [50], lowest value of “PRESS” and maximized Adjusted R^2 (0.9958) and Predicted R^2 (0.9659) [having a difference less than 0.2] among all above mentioned model [41,43,44].

Table III: FC-CCD data of three independent variables with their coded levels and response as percentage oil yields (% OY) of SCO_2E of clove buds

Run No.	Input Factors			Response
	ARSEF, $X_1 = \frac{r_o - r_i}{2L}$	Temperature, X2 (^{0} C)	Pressure,X3 (MPa)	%OY (g Oil/ 100 g Feed)
1	0.0283	45	19.6	16.42
2	0.0238	35	24.5	15.92
3	0.0283	40	19.6	16.07
4	0.0283	40	24.5	16.83
5	0.0327	35	24.5	13.51
6	0.0238	45	24.5	18.05
7	0.0327	40	19.6	14.55
8	0.0283	40	19.6	16.07
9	0.0283	40	19.6	16.07
10	0.0238	40	19.6	16.81
11	0.0327	45	14.7	14.13
12	0.0283	40	19.6	16.07
13	0.0283	40	14.7	15.03
14	0.0238	35	14.7	14.52
15	0.0238	45	14.7	15.79
16	0.0283	35	19.6	14.74
17	0.0327	45	24.5	15.85
18	0.0327	35	14.7	12.11
19	0.0283	40	19.6	16.07
20	0.0283	40	19.6	16.07

X1, ARSEF; X2, Extraction Temperature; X3, Extraction Pressure; %OY, Percent Oil Yield of Clove Buds

Finally, an ANNOVA test was performed for in-depth statistical studies of each process variables on the response for the selected quadratic model. The results of ANNOVA for response surface quadratic equation were illustrated in Table IV. High Model F-value of 503.32 suggested the response surface quadratic model is significant. P-values of the individual terms of the quadratic model equation less than 0.0500 indicate model terms are significant. Thus in this analysis X1, X2, X3, X1X2, X1X3, X2X3, $X1^2$, $X2^2$,$X3^2$ are all significant model terms.

On the other hand, the value of adequate precision, a measure of signal to noise ratio, greater than 4 is desirable towards perfect fitting. An adequate precision value of 95.881 obtained in ANNOVA indicates the selected quadratic model adequately described the true behaviour of the system in comparison to the linear model and 2FI models.

C. Model Equation obtained from RSM

The quadratic mathematical model representing the percentage oil yield (% OY) as clove buds within a function of the three independent variables of present study on SCO_2E of the range of their values under investigation can be expressed by the following generalised equation:

$$\%OY = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_3 X3 + \beta_{12} X1X2 + \beta_{13} X1X3 + \beta_{23} X2X3 + \beta_{11} X1^2 + \beta_{22} X2^2 + \beta_{33} X3^2$$

where %OY is the actual response; β_0 is the regression coefficient of intercept; β_1, β_2 and β_3 are the regression coefficients for linear fit; β_{12}, β_{13} and β_{23} are the regression coefficients for FI fit; and β_{11}, β_{22} and β_{33} are the regression coefficients for quadratic fit.

In terms of actual values of the regression coefficients the final regression model is given by:

$$\%OY = -31.02006 + (7.793\times10^{2}) X1 + 1.562 X2 + 0.289 X3 + 5.393 X1X2 - 3.096 X1X3 + (6.02\times10^{-3}) X2X3 - (20.888\times10^{3}) X1^2 - (20.545\times10^{-3}) X2^2 - (6.815\times10^{-3}) X3^2$$

Table IV: ANOVA for the clove buds SFE yield (% OY) in the FC-CCD

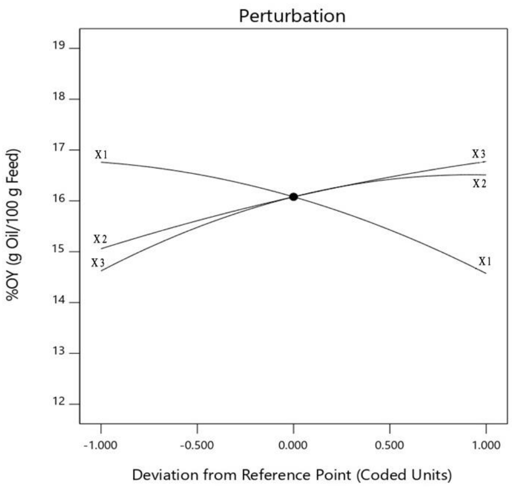
Source	Actual Regression Coefficients	Sum of Squares	df	Mean Square	F-value	p-value
Model		33.06	9	3.67	503.32	< 0.0001
X1	779.27810	11.97	1	11.97	1639.87	< 0.0001
X2	1.56208	8.91	1	8.91	1221.01	< 0.0001
X3	0.288898	7.36	1	7.36	1008.67	< 0.0001
X1 X2	5.39326	0.1152	1	0.1152	15.78	0.0026
X1 X3	-3.09562	0.0365	1	0.0365	4.99	0.0494
X2 X3	0.006020	0.174	1	0.174	23.85	0.0006
$X1^2$	-20888.088	0.4705	1	0.4705	64.47	< 0.0001
$X2^2$	-0.020545	0.7255	1	0.7255	99.41	< 0.0001
$X3^2$	-0.006815	0.0736	1	0.0736	10.09	0.0099
Residual		0.073	10	0.0073		
Lack of Fit		0.073	5	0.0146		
Pure Error		0.000	5	0.000		
Cor Total		33.13	19			
Std. Dev.	0.0854					
R^2	0.9978					
Adjusted R^2	0.9958					
Predicted R^2	0.9659					
Adeq Precision	95.8806					

X1, X2 and X3 relates the effects of main process variables ARSEF (r_o-r_i/2L), temperature (°C), and pressure (MPa), respectively on response (%OY). $X1^2$, $X2^2$ and $X3^2$ represents the quadratic affects of same input variables. X1X2, X1X3 and X2X3 are the interaction effects of three possible combinations of three variables (i) ARSEF and temperature; (ii) ARSEF and pressure , and (iii) temperature and pressure, respectively.

D. Effects of Bed Geometry, Temperature, and Pressure on the Oil Yield

Perturbation plot (Fig. 3) and two-factor interaction Response Surface plots Fig. 4(a-c) can be used to explain the effect of individual parameter as well as their interactions on the yield of extraction of clove buds in the range of values chosen for investigation. The Perturbation plot indicates the clove oil extracted by SCO_2E increases with increasing temperature from 35^0C-45^0C, initially at an increasing order from 35^0C-40^0C and after that with a decreasing order above 40^0C. This may be described by the counter effects of temperatures on solute and solvent of SFE. Temperature rise on one hand, improves the mass transfer rate of solute oil to the solvent phase by increasing diffusivity and vapour pressure values [51, 52, 40] and on the other hand, reduces the dissolving power of the solvent due to decreasing density. Temperature-solubility interferences are less significant at low temperature. At high temperature above it is more noticeable. Above 40^0C decreasing slope of %OY vs. temperature plot may be due to presence of this interference factor.

Similarly, Fig. 3 shows the pressure has an almost linear effect on oil yield at the range of pressure 14.7-24.5MPa [53]. P-value obtained from ANNOVA the diffusivity enhancing characteristics for the factor X3 representing extraction pressure was lowest and significant at 0.1% for linear fit as compared to the interactive and quadratic fits of X3 which were significant at 5%.



X1 : Bed Geometry Factor, X2 : Temperature (^{0} C),
X3 : Pressure (MPa)

Fig. 3. Effect of annular extractor bed geometry, temperature and pressure on the %OY of clove oil extracted by supercritical CO_2

This is well known that increase of pressure attributed to the increase of density of supercritical CO_2. With increasing density the interaction between solute molecules and CO_2 molecules increases due to decrease in intermolecular distances. Thus the dissolving power of CO_2 increases likewise liquid solvents and yield of oil increases [22, 54]. The adverse effects of increasing temperature on oil yields are insignificant up to 45^{0}C for clove buds [55].

Finally, the plot of %OY vs. factor X1 represents the influence of the modified annular extractor bed geometry over the conventional cylindrical bed type. Extract of SFE obtained from clove buds increases gradually and significantly from conventional bed to annular bed extractor containing larger size internal channel. Introduction of the annular feed bed should not oppose the pressure and temperature influences from their normal trends, rather increased the %OY for the same extraction time and same operating conditions. P-values of all linear, FI, and quadratic terms of X1 were significant in ANNOVA at 0.1%-5%.

The pressure–temperature interaction plot Fig.-4(a) shows increase in oil yield with increasing both the temperature as well as pressure in the range of 35-45^{0}C and 14.7-24.5KPa, respectively. Thus highest yield of clove buds by SFE was obtained at highest temperature –pressure point and lowest oil content was obtained at lowest point of the plot. It was observed that the solubility of bioactive components of essential oil depends on the balance between CO_2 density and vapour pressure of solute which in turn influenced by the extraction pressure and temperature. At low temperature and pressure the yield of oil increases with the increase of pressure at a given temperature due to less impact of temperature (up to about 45^{0}C) on solubility as compared to pressure [55]. Same way at low pressure the yield of oil increases with increasing temperature at a given pressure due to less effective negative impact of temperature on solubility as compared to more positive impact on vapour pressure and diffusivity [56].

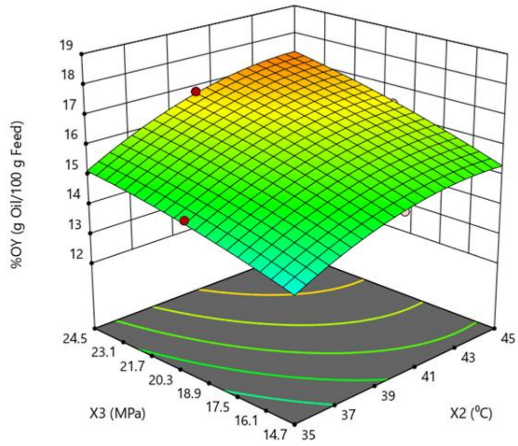


Fig. 4(a)

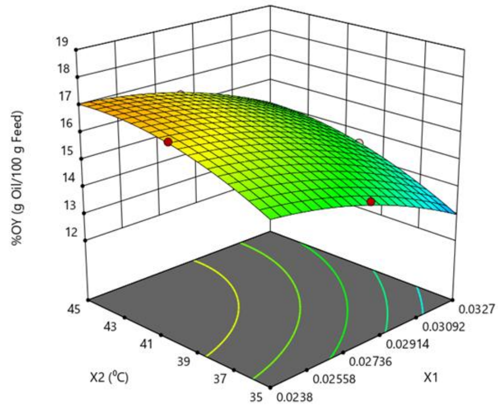


Fig. 4(b)

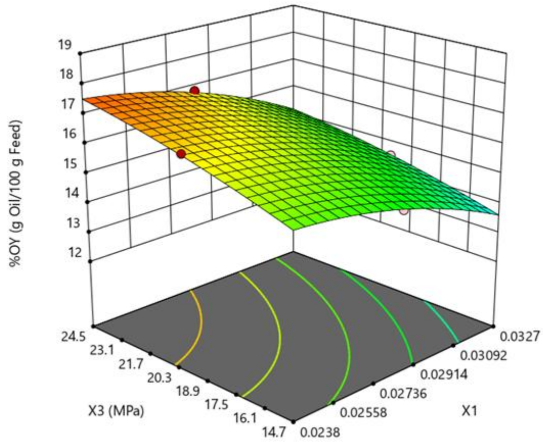


Fig. 4(c)

Fig. 4 Response surface plots (a-c) for clove oil:
Fig. 4(a) percent yield vs. extraction temperature and pressure at constant bed geometry (B2) ;
Fig. 4(b) percent yield vs. extraction temperature and bed geometry at constant pressure of 19.6MPa;
Fig. 4(c) percent yield vs. extraction pressure and bed geometry at constant temperature of 40°C

Fig. 4(b-c) show the three-dimensional plots of the response surfaces for the clove oil yield as related to extractor bed geometry parameter with temperatures as well as pressures. There, it is noticed that the increase of the ratio of radial to axial surface exhibit a positive effect on oil yield throughout the range of pressures and temperatures under this investigation. It is due to the fact that the increase of this ratio reduces the molecular diffusive path for both the solvent and solute molecules. This also induces turbulence in the fluid bulk that increases convective diffusion. As a result resistance to mass and heat transfer for oil extraction decreases yielding higher oil mass.

Fig. 5 represents the predicted response of ANNOVA vs. actual response in terms of %OY. Numerical optimization of the operating variables was carried out to predict the optimal condition in order to obtain the highest crude extraction yield of clove buds.

The optimum conditions were identified as 24.5MPa pressure, 44.72 °C temperature and bed type B3 with the optimal yield (%OY) of 17.981.

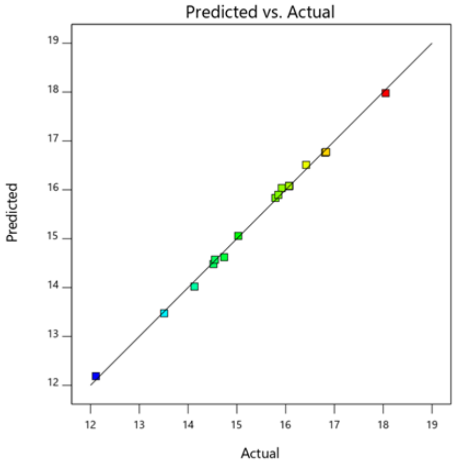


Fig. 5: Graphical representation of predicted response of %OY vs. actual response of %OY

E. Chemical Analysis of Essential Oil Components of Clove Buds

The chemical constituents of the clove oil obtained at 24.5MPa pressure, 45^0C temperature using bed type B3 by SCO_2E which was close to the optimum extraction condition of present RSM study were analyzed. The complete GC-MS chromatogram of clove oil sample is shown in Fig. 6. The components of clove essential oil were identified by comparing the retention times, mass fragmentation patterns of them with the available data of reference samples and GC-MS spectral database for organic compounds. The percent composition of essential oil constituents was determined using computerized normalization method from peak area of clove oil. Fig. 6 of chromatogram represents the presence several bioactive ingredients in the clove essential oil. The identified compounds present in the clove oil sample used for quantitative and qualitative analysis in this study were listed in Table V.

The main components identified in the clove extract were eugenol (72.08%), eugenyl acetate (11.84%), β-caryophyllene (6.73%), and caryophyllenoxide (3.06%). Roughly, the range of these constituents in good quality clove oil were reported as eugenol (70–95 %), eugenol acetate (up to 20 %) and β-caryophyllene (12–17 %) [33, 57, 58]. Main constituent eugenol percentage in this study match with the above mentioned range and indicates good quality oil. Presence of greater percentage of eugenol may be explained following the research work of Guan et. al., 2007 [22]. They reported in their work that the selective extraction of eugenol content is increased almost proportionally with temperature than other components. Clove oil analysed in this study was extracted using supercritical CO_2 at almost 45^0C which was almost 5^0C larger than the reported best extraction temperature by Prado et. al., 2011 [34].

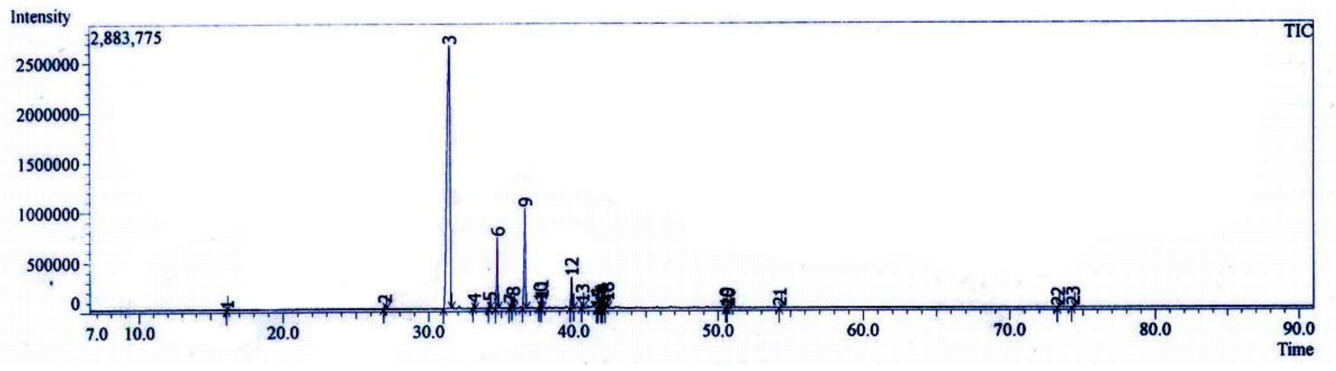


Fig. 6: Gas chromatogram of the constituents of essential oil extracted from clove buds

Table V: Percentage chemical composition of the volatile oil from clove buds

Compound Name	Mol. Wt.	Molecular Formula	Retention Time (Min)	% Conc.
P-Allylphenol	134	$C_9H_{10}O$	27.083	0.44
Eugenol NP	164	$C_{10}H_{12}O_2$	31.441	72.08
α-Copaene(23.49)	204	$C_{15}H_{24}$	33.106	0.38
α-Caryophyllene	204	$C_{15}H_{24}$	34.175	0.53
β-caryophyllene	204	$C_{15}H_{24}$	34.709	6.73
Humulene-(V1)	204	$C_{15}H_{24}$	35.583	0.29
Caryophyllene	204	$C_{15}H_{24}$	35.801	0.88
Eugenyl acetate	206	$C_{12}H_{14}O_3$	36.62	11.84
Calamenene(Trans) 29.96	202	$C_{15}H_{22}$	37.667	0.73
Delta-Cadinene	204	$C_{15}H_{24}$	37.757	0.52
Caryophyllenoxide	220	$C_{15}H_{24}O$	39.845	3.06
Humulene Epoxide	220	$C_{15}H_{24}O$	40.591	0.38
Cembrene	272	$C_{20}H_{32}$	41.888	0.30
2´,3´,4´ Trimethanoxyacetophenone	210	$C_{11}H_{14}O_4$	42.043	0.49
Caryophylenoxide	220	$C_{15}H_{24}O$	42.284	0.63

Rest components were present in the range of 0.03% - 0.24%.

F. Kinetics of Extraction under varying Extractor Bed Geometry:

Fig. 7(a-c) show that the variation in extractor bed geometry influenced the extraction rate while other conditions of extraction were maintained constant.

From the feasibility study on economic evaluation of some commercial SCO_2E process, it is considered that extraction of plant material will be continued up to recovery of 90% of the solute part. The increased operational cost involved with the continuation of extraction to recover rest portion of the volatile matter is not viable with the increments in the amount of product. In OEC, information about the CER period is very much important because it represents extraction period with highest and constant rate and greater productivity. Thus CER influences remarkably on the manufacturing cost of volatile matter from plant material. The largest amount of extract in unit time is possible in this CER period because solute mass is easily accessible by the solvent CO_2 and dissolved in supercritical phase. Table VI provides information about kinetic study parameters during CER period in terms of t_{CER}, R_{CER}, and Y_{CER}. For all the three levels of temperature (T1, T2, T3), OECs differed among beds B1 (representing conventional cylindrical bed geometry), B2 and B3 (representing annular extractor bed geometry of different dimensions.

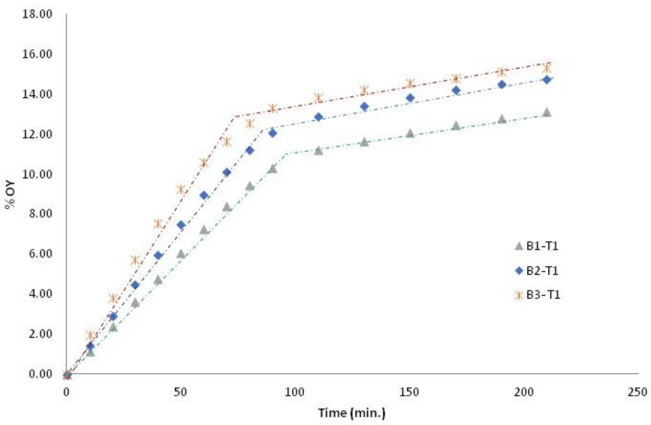


Fig. 7(a)

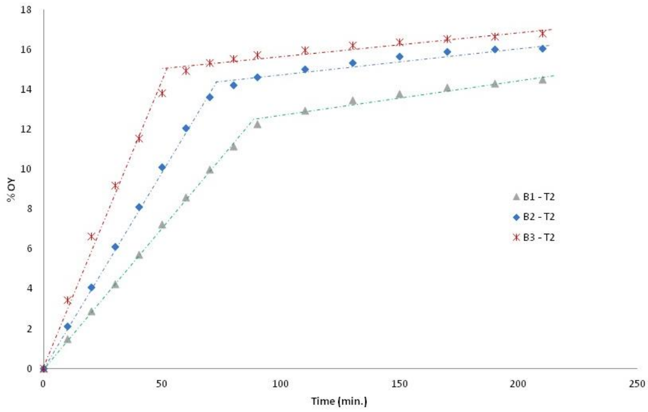


Fig. 7(b)

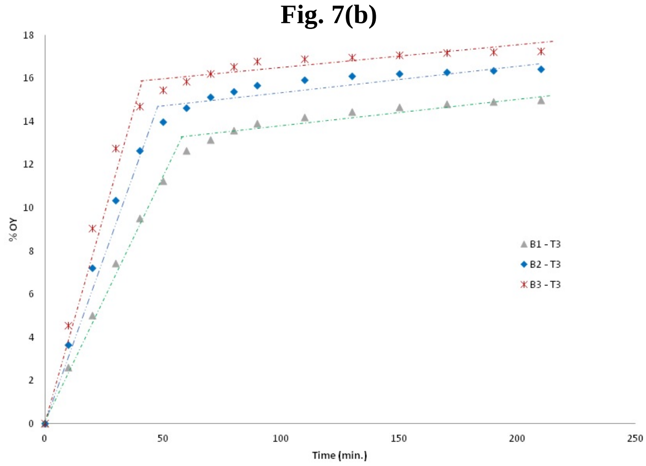


Fig. 7(c)

Fig. 7(a-c) Variation in OECs of clove buds oil under varying bed geometry B1, B2 and B3 for three different extraction temperatures of T1 (35^0C), T2 (40^0C), and T3 (45^0C), respectively.

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

In each bed with increasing temperature t_{CER} decreases, on the other hand R_{CER}, Y_{CER} and total yield (%OY) extracted in the period of 210 minutes increase. Positive effect of temperature on extraction of clove oil up to 45^0 C was reported in Guan et. al.,2007 research article [22]. Grosso et al. also reported that temperature helps to promote the faster release of the monoterpene hydrocarbons (main component of clove oil eugenol in this case) from the botanical materials [59]. It validates the increased values of R_{CER}, Y_{CER} during CER period. Comparative studies on the OEC curves of three beds reveal that t_{CER} is lowest in case of B3 type bed and highest in case of B1 type bed for all temperatures studied. On the other hand, R_{CER} and Y_{CER} are highest in case of B3 type bed and lowest in the case of B1 type bed for all temperatures studied. This happened due to the reduction of mass transfer resistance with the increase of the surface enhancement factor. As there is no depletion of solute molecules in the constant extraction rate period and surface diffusion controls the rate, the manifestation is quite prominent in CER rather than FER where pore diffusion controls the rate. From the rate curves it is also noted that though there increasing trends of R_{CER} and Y_{CER} from B1 to B3, it is not linear but of asymptotic in nature. This is quite obvious as larger voids invite ill effects of channeling.

Table VI: Experimental and estimated data for kinetic studies

Run	t_{CER} (min)	R_{CER} (10^{-6} kg/s)	Y_{CER} (%)	% OY (g Oil/ 100g Feed)
B1-T1	95.0	11.58	11.00	13.10
B2-T1	85.0	14.47	12.30	14.74
B3-T1	73.0	17.67	12.90	15.33
B1-T2	87.5	14.29	12.50	14.51
B2-T2	72.5	19.59	14.20	16.07
B3-T2	52.0	29.04	15.10	16.81
B1-T3	57.5	23.13	13.30	15.01
B2-T3	47.5	30.95	14.70	16.42
B3-T3	41.0	38.78	15.90	17.26

B1-T1, SCO_2E using extractor feed bed B1 with extraction temperature T1 (35^0C);
B2- T1, SCO_2E using extractor feed bed B2 with extraction temperature T1 (35^0C);
B3- T1, SCO_2E using extractor feed bed B3 with extraction temperature T1 (35^0C);
B1-T2, SCO_2E using extractor feed bed B1 with extraction temperature T2 (40^0C);
B2-T2, SCO_2E using extractor feed bed B2 with extraction temperature T2, (40^0C);
B3-T2, SCO_2E using extractor feed bed B3 with extraction temperature T2 (40^0C);
B1-T3, SCO_2E using extractor feed bed B1 with extraction temperature T3 (45^0C);
B2-T3, SCO_2E using extractor feed bed B2 with extraction temperature T3 (45^0C);
B3-T3, SCO_2E using extractor feed bed B3 with extraction temperature T3 (45^0C);
$t_{CER,}$ constant extraction rate period;
R_{CER}, the rate of extraction during CER;
Y_{CER},% yield achieved during CER;
%OY, percentage of total yield.

IV. CONCLUSION AND FUTURE SCOPE

The effects of bed geometry as axial to radial surface enhancement factor along with the common temperature-pressure parameters on the performance of supercritical carbon dioxide extraction of clove bud oil were studied and found to have definite advantages in terms of both the oil yields and time of extraction. The FC-CCD analysis of the results show that the clove oil extracted by SCO_2E increases with increasing temperature from 35^0C- 45^0C. But, the increasing tendency is not linear; rather initially it was of increasing order from 35^0C-40^0C and thereafter with a decreasing order. This may be due to the counter effect of temperatures on the solubility of solvent as the density of SCO_2 decreases with rising temperature.

The pressure has a positive effect on oil yield with almost linear with a slow rise in the range of pressures 14.7-24.5MPa. This is due to the increase of density of supercritical CO_2 with pressure and the interaction between solute molecules and CO_2 molecules increases with increasing density. Thus the dissolving power of CO_2 increases like conventional solvents and yield of oil increases. The effect of axial to radial surface area enhancement factor, $[(r_o-r_i)/2L]$, seems significant as extraction time reduces and oil yields increase with the increase of this factor. But after certain values of the enhancement factor, this became insignificant. This may be due to the fact that the channeling effect became pronounced at higher values of this enhancement factor.

Thus, it may be concluded that though axial to radial surface enhancement factor has a positive effect on SCO_2E, its efficacy after an optimal value gradually become flattened due to pronounce channeling effect. It is not clear at this stage, at least, how hydrodynamic behavior of the feed bed influences the extraction curve. Further systematic studies with different bed geometries in order to correlate hydrodynamic behavior are necessary for establishing a suitable criterion that can be used to predict extractor performance along with its economic gain.

ACKNOWLEDGEMENTS

This research work used the research facilities for supercritical fluid extraction developed under MODROB scheme of AICTE. The experimental works were financially supported by IIChE, Kolkata, India. GC-MS analysis of clove oil samples were done in the quality control laboratory of M/s Imperial Fragrances & Flavours Pvt. Ltd., Howrah, West Bengal, India.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ARSEF	Axial to radial surface enhancement factor
B	Bed type
CCD	Central composite design
CER	Constant extraction rate
D_P	Particle size
F	Mass of feed loaded
FC-CCD	Face centered central composite design

FER	Falling extraction rate
OEC	Overall extraction curve
OY	Percentage oil yield
Q_{CO2}	Solvent flow rate
R_{CER}	The rate of extraction during CER
RSM	Response surface methodology
SCO_2E	Supercritical fluid extraction technology using CO_2
SFE	Supercritical fluid extraction
SCO_2	Supercritical CO_2
T	Temperature
t_{CER}	Constant extraction rate period
t_E	Extraction time
t_S	Static period of extraction
Y_{CER}	% yield achieved during CER

REFERENCES

1. N. Margaris, A. Koedam and D. Vokou, "Aromatic Plants: basic and applied aspects," The Hague, London, Boston, Martinus Nijhoff Publishers, 1982.
2. R.K.M. Hay and P.G. Waterman (eds), "Volatile Oil Crops: Their Biology, Biochemistry and Production," Longman Scientific & Technical, Harlow, 1993, pp. 5-22.
3. S. Burt, "Essential oils: their antibacterial properties and potential applications in Foods," International Journal of Food Microbiology, Vol.94, 2004, pp. 223-253.
4. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M, Radice, and R. Bruni, "Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods," Food Chemistry 91, 2005, pp. 621–632.
5. F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar, "Biological effects of essential oils – a review," Food and Chemical Toxicology 46, 2008, pp. 446–475.
6. M. Hunter, "Essential Oils: Art, Agriculture, Science," New York: Industry and Entrepreneurship, Nova Science Publishers, Inc., 2009, pp. 43-63.
7. Capuzzo, M. E. Maffei and A. Occhipinti, "Supercritical Fluid Extraction of Plant Flavors and Fragrances," Molecules 2013, 18, pp. 7194-7238.
8. Ali, N. AliAl-Wabel, S. Shams, A. Ahamad, S.A. Khan, F. Anwar, "Essential oils used in aromatherapy: A systemic review" Asian Pacific Journal of Tropical Biomedicine , vol.5, Issue 8, August 2015, pp. 601-611.
9. J.R. Calo, P.G. Crandall and C.A.O. Bryan, "Essential oils as antimicrobials in food systems – A review," Food Control Volume 54, 2015, pp. 111-119.
10. C.M. Cook and T. Lanaras, "Essential Oils: Isolation, Production and Uses," Encyclopedia of Food and Health, 2016, pp. 552-557.
11. M. Chellappandian, P. Vasantha-Srinivasan and S. Senthil-Nathan, "Botanical essential oils and uses as mosquitocides and repellents against dengue," Environment International, vol. 113, 2018, pp. 214-230.
12. K.H.C. Baser and G. Buchbauer, "Handbook of Essential Oils: Science, Technology, and Applications," CRC Press, October 27, 2015.
13. Barbieri and P. Borsotto, "Essential Oils: Market and Legislation," Potential of Essential Oils, September'2018.
14. K.U. Sankar, "Supercritical fluid carbon dioxide technology for extraction of species and other high value bio-active compounds," In: S.S.H. Rizvi (ed) Supercritical fluid processing of food and biomaterials, Blackie, London, 1994, pp. 155–167.
15. Fadel, F. Marx, A. El-Sawy and A. El-Ghorab, "Effect of extraction techniques on the chemical composition and antioxidant activity of Eucalyptus camaldulensis var. brevirostris leaf oils," Z Lebensm Unters Forsch A, 1999, 208, pp: 212–216.
16. Zizovic, M. Stameni´c, J. Ivanovi´c, A. Orlovi´c, M. Risti´c, S. Djordjevi´c, S.D. Petrovi´c and D. Skala, "Supercritical carbon dioxide extraction of sesquiterpenes from valerian root," *Journal Supercritical Fluids* 43, 2007, pp. 249–258.
17. H.A. Martinez-Correa, P.M. Magalhães, C.L. Queiroga, C.A. Peixoto, A.L. Oliveira and F.A. Cabral, "Extracts from pitanga (Eugenia uniflora L.) leaves: influence of extraction process on antioxidant properties and yield of phenolic compounds," *Journal Supercritical Fluids* 55, 2011, pp. 998–1006.
18. Gracia, J.F. Rodríguez, A. De Lucas, M.P. Fernandez-Ronco and M.T. García, "Optimization of supercritical CO_2 process for the concentration of tocopherol, carotenoids and chlorophylls from residual olive husk," *Journal Supercritical Fluids* 59, 2011, pp. 72–77.
19. M. Bimakra, R.A. Rahmana, F. S. Taipa, A. Ganjloob, L.M. Salleha, J. Selamatc, A. Hamidc and I.S.M. Zaidulc, "Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) Leaves", Food And Bioproducts Processing 89, 2011, pp. 67–72.
20. Ivanovica, S. Dimitrijevic-Brankovicb, D. Misicc, M. Ristict and I. Zizovica, "Evaluation and improvement of antioxidant and antibacterial activities of supercritical extracts from clove buds," Journal of Functional Foods, 5, 2013, pp. 416-423.
21. Herrero, J.A. Mendiola, A. Cifuentes and E. Ibanez, "Supercritical fluid extraction: Recent advances and applications," Journal of *Chromatography* A, 1217, *2010, pp. 2495–2511*.
22. Wenqiang, L. Shufen, Y. Ruixiang, T. Shaokun and Q. Can "Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods," Food Chemistry 101, 2007, pp.1558–1564.
23. T.M. Takeuchi, P.F. Leal, R. Favareto, L. Cardozo-Filho, M.L. Corazza, P.T.V. Rosa and M.A.A. Meireles, "Study of the phase equilibrium formed inside the flash tankused at the separation step of a supercritical fluid extraction unit," Journal of Supercritical Fluids 43, 2008, pp. 447–459.
24. Sosova and R.P. Stateva, "Supercritical fluid extraction from vegetable materials," Reviews in Chemical Engineering 27, 2011, pp. 79–156.
25. J.A. Rocha-Uribe, J.I. Novelo-Pérez, C.A. Ruiz-Mercado," Cost estimation for CO2 supercritical extraction systems and manufacturing cost for habanero chilli", The Journal of Supercritical Fluids 93, 2014, pp. 38-41.
26. Priyanka, S. Khanam, "Influence of operating parameters on Supercritical fluid extraction of essential oil from Turmeric root," Journal of Cleaner Production, 188, 2018, pp. 816-824.
27. Angela and A. Meireless, "Supercritical extraction from solid: process design data," Current opinion in solid state and material science, Elsevier Ltd., 7, 2003, pp. 321–330.
28. G.L. Zabot, M.N. Moraes and M.A.A. Meireles, "Supercritical Fluid Extraction of Bioactive Compounds from Botanic Matrices: Experimental Data, Process Parameters and Economic Evaluation", Recent Patents on Engineering 6, 2012, pp. 182-206.
29. G.L. Zabot, M.N. Moraes, A.J. Petenateb and M.A.A. Meireles, "Influence of the bed geometry on the kinetics of the extraction of clove bud oil with supercritical CO2," The Journal of Supercritical Fluids 93, 2013, pp. 56-66.
30. G.L. Zabot, M.N. Moraes and M.A.A. Meireles, "Influence of the bed geometry on the kinetics of rosemary compounds extraction with supercritical CO2," The Journal of Supercritical Fluids 94, 2014, pp. 234–244.
31. G.A. Núñez and J.M. dell Valle, "Supercritical CO2 oilseed extraction in multi-vessel plants. 2. Effect of number and geometry of extractors on production cost," The Journal of Supercritical Fluids 92, 2014, pp. 324–334.
32. E.J. Bowles, "The Chemistry of Aromatherapeutic Oil," 3rd Edition, Griffin Press, 2003.
33. Gopalakrishnan, C.S. Narayanan and A.G. Mathew, "Chemical composition of Indian clove bud, stem and leaf oils," India Perfume, 32, 3, 1988, pp. 229 -235.
34. J.M. Prado, G.H.C. Prado and M.A.A. Meireles "Scale-up study of supercritical fluid extraction process for clove and sugarcane residue", The Journal of Supercritical Fluids 56, 2011, pp. 231-237.
35. M. Parle and D. Khanna, "*Clove:A Champion Spice," International Journal of Research in Ayurveda and Pharmacy 2(1), 2011, pp. 47-54.*
36. Bhowmik, K.P.S. Kumar, A. Yadav, S. Srivastava, S. Paswan and A.S. Dutt, " Recent Trends in Indian Traditional Herbs Syzygium aromaticum and its Health Benefits," Journal of Pharmacognosy and Phytochemistry 1, 2012, pp.13-22.
37. Chaieb, H. Hajlaoui, T. Zmantar, A.B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, "The chemical composition and biological activity of clove essentialoil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review," Phytotherapy Research 21, 2007, pp. 501–506.
38. J.W. Goodrum and M.B. Kilgo, "Peanut oil extraction with SC-CO2: Solubility and kinetic functions," Transactions of the ASAE, 30(6), 1987, pp. 1865-1868.
39. Ivanovic, M. Ristic and D. Skala, "Supercritical CO2 extraction of Helichrysum italicum: Influence of CO2 density and moisture content of plant material," The Journal of *Supercritical Fluids* 57, 2011, pp. 129-136.

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

40. A. Rai, B. Mohanty and R. Bhargava, “Modelling and response surface analysis of supercritical extraction of watermelon seed oil using carbon dioxide”, Separation and Purification Technology, 141, 2015, pp. 354-365.
41. C. Montgomery, “Design and analysis of experiments” (5th ed.), New York, Wiley, 2001.
42. O. Haaland, “Experimental design in biotechnology,” New York, Marcel Dekker, 1989.
43. R. H. Myers and D.C. Montgomery, “Response surface methodology: Process and product optimization using designed experiments” (2nd ed.). New York: Wiley. 2002.
44. Liyana-Pathirana, and F. Shahidi, “Optimisation of extraction of phenolic compounds from wheat using response surface methodology,” Food Chemistry, 93, 2005, pp. 47–56.
45. Bimakr, R.A. Rahman, A. Ganjloo, F.S. Taip, L.M. Salleh and M.Z.I. Sarker, “Optimization of Supercritical Carbon Dioxide Extraction of Bioactive Flavonoid Compounds from Spearmint (Mentha spicata L.) Leaves by Using Response Surface Methodology,” Food Bioprocess Technolology 5, 2012, pp. 912–920.
46. Xu, X. Zhan, Z. Zeng, R. Chen, H. Li, T. Xie and S. Wang, “Recent advances on supercritical fluid extraction of essential oils”, African Journal of Pharmacy and Pharmacology 5(9), 2011, pp. 1196-1211.
47. P.C. Frohlicha, K.A. Santosa, F. Palúa, L. Cardozo-Filhob, C. Silvab and E.A. Silvaa, “Evaluation of the effects of temperature and pressure on the extraction of eugenol from clove (Syzygium aromaticum) leaves using supercritical CO2,” The Journal of Supercritical Fluids 143, 2019, pp. 313-320.
48. Mukhopadhyay and K. Rajeev, “Parametric study and mass transfer: modelling of super-critical CO2 extraction of clove,” Ind. Chem. Eng. Trans., 1, 742,1998.
49. L. Wang, B. Yang, X. Du and C. Yi, “Optimisation of supercritical fluid extraction of flavonoids from Pueraria lobata,” Food Chemistry 108, 2008, pp. 737–741.
50. H. N. Sin, S. Yusof, N. Hamid, and R.A. Rahman, “Optimisation of enzymatic clarification of sapodilla juice using response surface methodology,” Journal of Food Engineering, 73, 2006, pp. 313–319.
51. K.L. Nyam, C.P. Tan, O.M. Lai, K. Long and Y.B.C. Man, “Optimization of supercritical 35 CO2 extraction of phytosterol-enriched oil from Kalahari melon seeds,” Food and Bioprocess Technology, 4, 2011, 1432-1441.
A. Terada, N. Kitajima, S. Machmudah, M. Tanaka, M. Sasaki and M. Goto, “Cold-pressed yuzu oil fractionation using countercurrent supercritical CO2 extraction column,” Separation and Purification Technology, 71, 2010, pp. 107-113.
52. X. Xu, Y. Gao, G. Liu, Q. Wang and J. Zhao, “Optimization of supercritical carbon dioxide extraction of sea buckthorn (Hippophaë thamnoides L.) oil using response surface methodology,” *LWT - Food Science* and *Technology* 41, 2008, pp.1223-1231.
53. M. S. Liza, R.A. Rahman, B. Mandana, S. Jinap, A. Rahmat, I. S. M Zaidul and A. Hamid, “Supercritical carbon dioxide extraction of bioactive flavonoid from Strobilanthes crispus (Pecah Kaca),” Food and Bioproducts Processing, 88, 2009, pp. 319–326.
54. S. Zhang, Y.G. Zu, Y.J. Fu, M. Luo, W. Liu, J. Li and T. Efferth, “Supercritical carbon dioxide extraction of seed oil from yellow horn (Xanthoceras sorbifolia Bunge.) and its anti-oxidant activity,” Bioresource Technology, 101, 2010, pp. 2537-2544.
55. L. Wang , C.L. Weller , V.L. Schlegel , T.P. Carr and S.L. Cuppett, “Supercritical CO2 extraction of lipids from grain sorghum dried distillers grains with soluble,” Bioresource Technology, 99(5), 2008, pp.1373-82.
56. L. Jirovetz, G. Buchbauer, I. Stoilova, A. Stoyanova, A. Krastanov, E. Schmidt, “Chemical composition and antioxidant properties of clove leaf essential oil,” Journal of Agricultural and Food Chemistry, 54(17), 2006, pp. 6303–6307.
57. Nurdjannah, N. Bermawie, “Handbook of Herbs and Spices” (Second Edition), Volume 1, 2012.
58. Grosso, V. Ferraro, A. Figueiredo, J. Barroso, J. Coelho, A. Palavra, “Supercritical carbon dioxide extraction of volatile oil from Italian coriander seeds,” Food Chemistry, 111, 2008, pp. 197-203.

AUTHORS PROFILE

Sutapa Roy, is currently working as Assistant Professor in the Department of Chemical Engineering in Haldia Institute of Technology. She completed her M.ChE from Jadavpur University and currently perusing PhD on supercritical fluid extraction. She has published several papers in national, international journal and conferences.

Dr. Chandan Guha, is a professor of Department of Chemical Engineering, Jadavpur University, India. He has over 30 years of teaching experience. His specialization is transport phenomena, numerical heat transfer, process modeling and simulation, computational fluid dynamics, reaction engineering and computational two phase flow. He has published about 50 journal and conference papers. His research areas are melting-solidification process, moving boundary problems, loss of coolant accident problems, compressible flow etc. He authored two books on transport phenomena and chemical reaction engineering. He supervised 12 doctoral thesis. He has chaired many sessions in national and international conference. He has delivered about 60 invited lectures. He also served as Head of the Department and different academic and administrative bodies of Jadavpur University. He is also a reviewer of different journals.

Asit Kumar Saha, is currently Professor in Chemical Engineering and the Principal of Haldia Institute of Technology. He obtained his BE degree from Jadavpur University, Kolkata and M.Tech and PhD degrees from Indian Institute of Technology, Kharagpur. Since 1993 he has been engaged in teaching and research. He has authored several papers published in national and international refereed journals.

Somak Jyoti Sahu, received his M. Tech and PhD degree from Jadavpur University and is working as Associate Professor at Haldia Institute of Technology, Haldia. His research areas include Process Modeling, Simulation, Control and reaction engineering. He has published several papers in national, international journals and conference.