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Optimization of Supercritical Carbon Dioxide Extraction of Phenolic Compounds from Mango Ginger Rhizome (*Curcuma Amada* Roxb.) Using Response Surface Methodology

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Abstract Relying on modern methods of separation, strict quality control, advanced technology and optimal manufacturing process, supercritical carbon dioxide (SC-CO_2) is considered to offer an opportunity to efficiently and economically improve recovery and reproducibility of natural product in its pure form. Three-level Box–Behnken factorial design (BBD) from response surface methodology (RSM) was applied to optimize the main extraction conditions including pressure, temperature and extraction time. The three different levels of Pressure, temperature and time are between 100-250 bar, 40-60°C and 5-15 hrs respectively. The optimum conditions were found to be 350 bar, 60°C and 15 hrs. Under the optimum conditions, total phenolic content obtained was 152 mg GAE/total extract, which well agreed with the predicted yield. HPLC analysis was carried out for the optimum conditions for the identification and quantification of the phenolic compounds.

Keywords: *Supercritical carbon dioxide, mango ginger, phenolic compounds, response surface methodology*

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1. Introduction

Curcuma amada Roxb popularly known as mango ginger belongs to the family Zingiberaceae is widely cultivated in India apart from Malaysia, China, Bangladesh, Myanmar, Thailand, Japan and Australia. It is a unique spice morphologically similar to ginger but, imparts mango flavour [1] and characteristics odour of rhizome is due to terpene hydrocarbons cis-ocimene and car-3-ene [2]. They are extensively used in Indian subcontinent for the preparation of culinary items like pickles, sauces etc, because of their exotic aroma. In Ayurveda, a traditional system of medicine in India, the plant is given more importance as an appetizer, alexiteric, antipyretic, aphrodisiac and laxative [3]. The essential oil of rhizome exhibited antimicrobial, antifungal and anthelmintic activity against tape worms [4].

In recent years there is an increasing demand for nutraceuticals from natural sources because of several advantages such as fever, side effects, better patient tolerance, relatively low price and acceptance due to a long history of use [5]. These naturaceuticals, ex. phenolic compounds, flavanoids, carotenoids, tocopherols etc belonging to different classes of chemicals can be obtained by several extraction techniques [6]. Conventional solvent extraction of phytochemicals has distinct drawbacks due to long extraction times, labor intensive operations, high cost of energy, handling of large volumes of hazardous solvents requiring concentration steps that can result in toxic solvent residues and degradation of valuable components due to heat [7,8]. Supercritical Fluid Extraction (SCFE) is widely used as an alternative technique for the extraction of nutraceuticals. The advantages of using SC-CO_2 compared to organic solvents are that CO_2 is nontoxic, non flammable and noncorrosive and in addition, it is cheap and readily available with a high degree of purity. In processing terms, CO_2 has a low critical temperature and pressure (31.2°C and 72.8 bar), which makes it the ideal solvent for natural products, since they do not undergo thermal degradation during the process. The Solvent can be removed and recovered from the extracts after processing by simply returning to atmospheric temperature [9,10,11,12].

Optimization of extraction parameter is one of the main aspects that should be considered in supercritical fluid extraction (SFE) to enhance the extraction and recovery of target compound. Despite of the valuable information obtained by phase equilibrium engineering, it is a common practice to optimize the processes using experimental designs and statistical modeling [13,14]. Response surface methodology (RSM) is a popular and useful statistical technique which has been applied in research to study complex variable processes. This experimental methodology combines mathematics with statistics for generating a mathematical model to describe the process, analyzing the effects of the independent variables and optimizing the processing operations [15].

The objective of this chapter is to (i) employ a Box-Behnken design (Box and Behnken, 1960) to assess the effect of variables like temperature, pressure and time on the amount of total extraction yield and total phenolic content from mango ginger powder and finding the optimum conditions for maximum yield of total extract and Total Phenolic Content (ii) characterization of phenolic acids obtained at optimum conditions.

2. Materials and Method

2.1. Experimental Material

Fresh and matured mango ginger rhizome was procured from local market, Mysore, Karnataka, India. The moisture content of fresh rhizome was estimated by toluene distillation method and found to be 90 ± 0.5 % (wb). The rhizomes were washed to remove adhered soil and sliced using a slicing machine (M/s Robot coupe, USA, Model: CL 50 Gourmet). The slices were dried at 45 ± 2°C in Low temperature Low humidity (LTLH) dryer. The dried material was ground in hammer mill (M/s Apex, USA) and the mean particle diameter was 480 ± 40 µm as measured by Particle size analyzer (Model: CIS-100, M/s Galai production, Israel).

2.2. Chemicals

Food grade CO_2 (99.99% pure) was used as solvent for extraction supplied by Ms. Kiran Corporation, Mysore, Karnataka, India. Gallic acid, tannic acid, caffeic acid, protucatuchuic acid, p-comaric acid, cinnamic acid, ferulic acid, syringic acid were purchased from Sigma-Aldrich Fine chemicals (St. aouis, MO, USA). HPLC grade water, methanol and acetic acid were obtained from Merk, Mumbai, India. Folin-Ciocalteu reagent was from Loba Chemie (Mumbai, India). All other chemicals used were of analytical grade.

2.3. Supercritical Carbon-Dioxide Extraction

High-pressure SCF equipment (NOVA Swiss WERKE AG, EX 1000-1.4-1.2 type, Switzerland) designed to working pressures of up to 1000 bar and temperature up to 100°C was used for all the extractions (Figure 1). The mango ginger powder was loaded into the extraction vessel which is of 1.1 lit in capacity. A Set experiments were conducted at different pressures (100-350 bar), temperature (40-60°C), and extraction time (5-15 hr). After attaining the desired temperature the CO2 which had been compressed to the set pressure was allowed into the extractor. A fraction has been collected from the separator at definite time intervals and the weight of the extract would be noted. The average flow rate was maintained around 1.8-2.0 kg/hr.

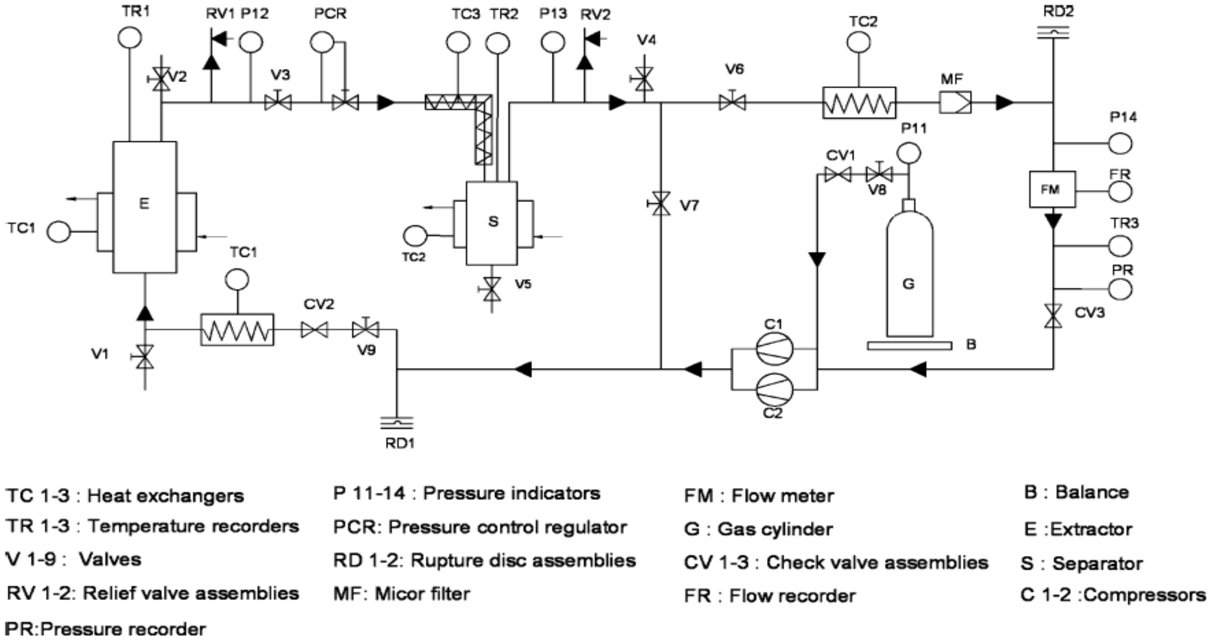

Figure 1. Schematic flow diagram of SC-CO2 extraction

2.4. Phenolic acid analysis

2.4.1. Determination of Total Phenolic Content (TPC)

Total phenolic content of SCF-MG extracts were measured according to Folin-Dennis method [16]. 200 µg of SCF-MG extract was dissolved in 500 µl of methanol and the sample was incubated with 2.5 ml of 10% Folin and Ciocalteu's (FC) phenol reagent for 2 min at room temperature. To this 2 ml of 7.5% Na_2CO_3 was added and incubated for 1 hr at ambient temperature. The absorbance was measured at 765 nm against a blank developed with 0.5 ml solvent using a Shimadzu UV-Visible spectrophotometer and gallic acid was used as standard. Total phenolic content in the extract was expressed as gallic acid equivalents (GAE).

2.4.2. High Performance Liquid Chromatography for Phenolic Acids

The bioactive phenolic acids of Mango ginger SCF extracts were analyzed by HPLC (model LC-10A, Shimadzu Corp.) on a reversed phase Shimpak C_{18} column (4.6 x 250 mm, Shimadzu Corp.) using a diode array UV detector (operating at λ_{max}) 280 nm). The mobile phase was composed of water/acetic acid/methanol (80: 5: 15 v/v/v). The isocratic chromatography was run at flow rate of 1 mL/min. Phenolic acid standards such as caffeic, coumaric, cinnamic, ferulic, gallic, gentisic, protocatechuic, syringic, and vanillic acids were employed for the identification of phenolic acids present in SCF-Mango ginger by comparing the retention time under similar experimental conditions [17].

2.5. Design of Experiments

The effect of the operating variables on the extraction yield and total phenolic content was studied using Box–Behnken design of response surface methodology. The experimental design used to investigate the effect of the factors were pressure (X_1), temperature (X_2) and time (X_3) with three levels for each variable, while the dependent variable was the extract yield (Y) or Total Phenolic Content (TPC). Codes and levels of independent variables of Pressure, temperature and time in RSM design were given in Table 1.The predicted values of total extract yield were calculated during regression model and compared with the experimental values. A second order quadratic polynomial equation was used as the regression model for each response (Eq. 1)

$$\begin{aligned}\mathbf{Y} = \mathbf{A}_0 + \mathbf{A}_1\mathbf{X}_1 + \mathbf{A}_2\mathbf{X}_2 + \mathbf{A}_3\mathbf{X}_3 \\ + \mathbf{A}_4\mathbf{X}_1^2 + \mathbf{A}_5\mathbf{X}_2^2 + \mathbf{A}_6\mathbf{X}_3^2 + \mathbf{A}_7\mathbf{X}_1\mathbf{X}_2 \\ + \mathbf{A}_8\mathbf{X}_1\mathbf{X}_3 + \mathbf{A}_9\mathbf{X}_2\mathbf{X}_3 + \boldsymbol{\varepsilon}\end{aligned} \tag{1}$$

Where Y is the predicted extract yield or TPC, A_0 is the intercept, X represents the independent variable (pressure, temperature and time) and A_1-A_3 are linear coefficients, A_4-A_6 are quadratic coefficients, A_7-A_9 are interaction coefficients and ε random error respectively. Coefficients for the above equation and Analysis of variance (ANOVA) were determined by employing Microsoft Excel data analysis tool. Three-dimensional response contour plots were obtained using the Kyplot software version 2.0 beta by varying any two of the variables and maintaining the other variable at + 1 coded value. Search for optimum yield within the range of variables was carried using SOLVER function of the MS-Excel-2010®software [7,18].

Table 1. Codes and levels of independent variables of pressure, temperature and time in RSM design

Symbols	Independent Variable	Coded levels		
		-1	0	1
X_1	Pressure (bar)	100	225	350
X_2	Temperature(°C)	40	50	60
X_3	Time (hr)	5	10	15

3. Result and Discussion

3.1. Effect of Pressure, Temperature and Time on the Extraction Yield and Total Phenolic Content

The solvating power of the supercritical fluid varies significantly as the variation in the applied temperature and pressure. Because density is the function of pressure and temperature, as the density increases the fluid behaves like a liquid and solubility will enhance. Depending upon the pressure and temperature, the yield of extract increased with increasing in CO2 mass and time. At constant temperature, the yield of extract increased with the increase in pressure. This happens due to increase in density of CO2 and intermolecular physical interactions with increasing pressure at constant temperature. At constant pressure, the yield decreases with increase in temperature because of decrease in the solvent density. This trend was followed at tower pressures. However, despite of the increase in temperature the yield of the extract increased at higher pressure (350 bar) due to increase in the vapor pressure of the active components in the extract. An increase in temperature could accelerate the mass transfer of solute in the matrix and/or from the matrix to the solvent thus increasing the extraction yield [19].

Phenolic content was calculated from Spectrometric method (FC method) and expressed in Gallic acid equivalents per total extract obtained at the specific extraction condition. Amount of phenolic content in the extract also follow the same pattern of the extraction yield with changing in the process parameters. In the present investigation the solute vapor pressure dominates solvent density. Similar results were noticed when extraction time was kept constant. As temperature increased, TPC decreased at pressure less than 250 bar and above 250 bar the trend reverses.

3.2. Statistical Analysis

Table 2. Regression Statistics of the response surface model

	Regression Statistics	
	TPC	**Yield**
Coefficient of Correlation (R)	0.927	0.93
Standard Error	26.62	0.479
Observations	15	15

All 15 of the designed experiments were conducted according to the Box-Bhenken design and the results were analyzed by multiple regression analysis using Microsoft office excel 2010 data analysis tool pack. Two responses were considered i.e yield and total phenolic content. The regression equation includes liear, quadratic and polynomial terms. The Box-Behnken design showed that polynomial regression models were in good agreement with the experimental results. The model was built based on the variables with confidence levels of 95%. The regression statistics and analysis of variance (ANOVA) for yield and total phenolic content reported in Table 2-Table 4. Indicates that the model is highly significant, which is evident from the high Fisher (F) ratio value. The coefficients of correlation (R) 0.927 and 0.93 for TPC and yield respectively indicates a high degree of correlation between observed and predicted values (Figure 2) thus indicating adequacy of the fitted models. Figure 3a-b show the effect of P, T and time on the total phenolic content, at constant temperature and as the pressure increases there is increase in the TPC (Table 5).

Table 3. Analysis of variance for yield and TPC

	ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*
TPC	**Regression**	9	21864.33	2429.37	3.42	0.094
	Residual	5	3543.922	708.78		
	Total	14	25408.25			
Yield	**Regression**	9	7.38	0.82	3.56	0.087
	Residual	5	1.15	0.23		
	Total	14	8.53			

Table 4. Regression coefficient of polynomial functions of response surface of total extract yield and total phenolic content

		Total Phenolic Content				Yield			
		Coefficients	SE	t Stat	P-value	Coefficients	SE	t Stat	P-value
	A_0	636.00	400.79	1.586	0.1734	10.446	7.220	1.446	0.207
X_1	A_1	-0.6986	0.703	-0.99	0.3657	-0.0239	0.012	-1.895	0.116
X_2	A_2	-23.503	14.341	-1.64	0.1621	-0.3505	0.258	-1.356	0.232
X_3	A_3	22.395	18.070	1.240	0.2702	0.347	0.325	1.066	0.335
X_1^2	A_4	-0.0019	0.0009	-2.167	0.0824	-7.5E-06	1.6E-05	-0.472	0.656
X_2^2	A_5	0.14919	0.1385	1.077	0.3307	0.00233	0.002	0.935	0.392
X_3^2	A_6	-0.7814	0.5542	-1.410	0.2175	-0.0037	0.0099	-0.371	0.725
$X_1.X_2$	A_7	0.03535	0.0106	3.320	0.0210	0.00063	0.0001	3.290	0.021
$X_1.X_3$	A_8	-0.0034	0.0212	-0.162	0.8772	2.99E-05	0.0003	0.078	0.940
$X_2.X_3$	A_9	-0.0300	0.2662	-0.112	0.9145	-0.00368	0.0047	-0.767	0.4772

Table 5. Experimental and predicted data for the yields and TPC obtained from Box–Behnken design

	Coded value			**Uncoded value**			**Response**					
Run	**X_1**	**X_2**	**X_3**	**Pressure**	**Temperature**	**Time**	**Y_e**	**Y_p**	**ε_Y**	**TPC_e**	**TPC_p**	**ε_{Tpc}**
1	-1	1	0	100	60	10	0.213	0.049	0.164	6.17	10.33	-4.15
2	-1	-1	0	100	40	10	1.365	1.865	-0.49	87.92	117.28	-29.35
3	-1	0	-1	100	50	5	0.415	0.172	0.242	23.36	4.76	18.59
4	1	-1	0	350	40	10	1.243	1.407	-0.16	75.51	71.36	4.15
5	0	0	0	225	50	10	1.404	1.402	0.002	100.15	100.15	-7E-14
6	0	-1	1	225	40	15	2.728	2.322	0.406	143.19	128.74	14.44
7	0	0	0	225	50	10	1.396	1.402	-0.005	100.15	100.15	-7E-14
8	0	0	0	225	50	10	1.405	1.402	0.003	100.15	100.15	-7E-14
9	1	0	1	350	50	15	2.005	2.247	-0.24	73.48	92.08	-18.59
10	0	1	-1	225	40	5	1.257	1.000	0.257	91.64	80.88	10.76
11	0	1	1	225	60	15	1.459	1.717	-0.25	96.41	107.17	-10.76
12	0	1	-1	225	60	5	0.725	1.131	-0.406	50.87	65.32	-14.44
13	1	1	0	350	60	10	3.248	2.748	0.499	170.55	141.19	29.35
14	-1	0	1	100	50	15	1.182	1.089	0.093	68.85	53.94	14.91
15	1	0	-1	350	50	5	1.162	1.255	-0.093	36.64	51.56	-14.91
Optimum	-	-	-	350	60	15	2.835	2.967	-0.132	153.93	145.69	-8.234

X_1-Pressure, bar **X_2**-Temperature, °CX_3-Time, hr **Y_e**-Experimental yield, % **Y_p**-Predicted yield, %ε_Y-residuals (Y_e-Y_p), % **TPC_e**-Experimental Total Phenolic content, mg of GAE/total extract **TPC_p**-Predicted Total Phenolic content, mg of GAE/total extract, **ε_{TPC}**-residuals (Y_{TPC}-Y_{TPC}), mg of GAE/total extract

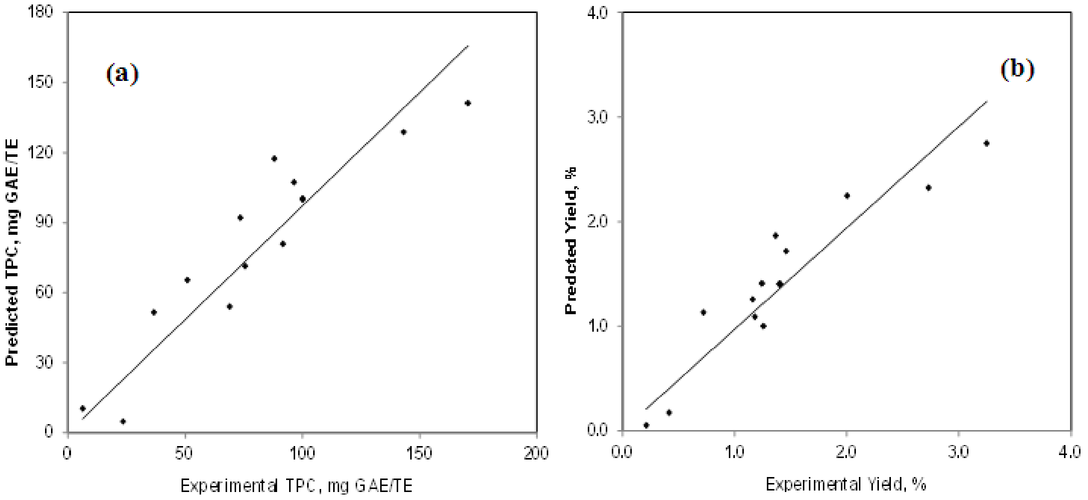

Figure 2. Plot of experimental values vs model predicted values (a) TPC, (b) Yield %

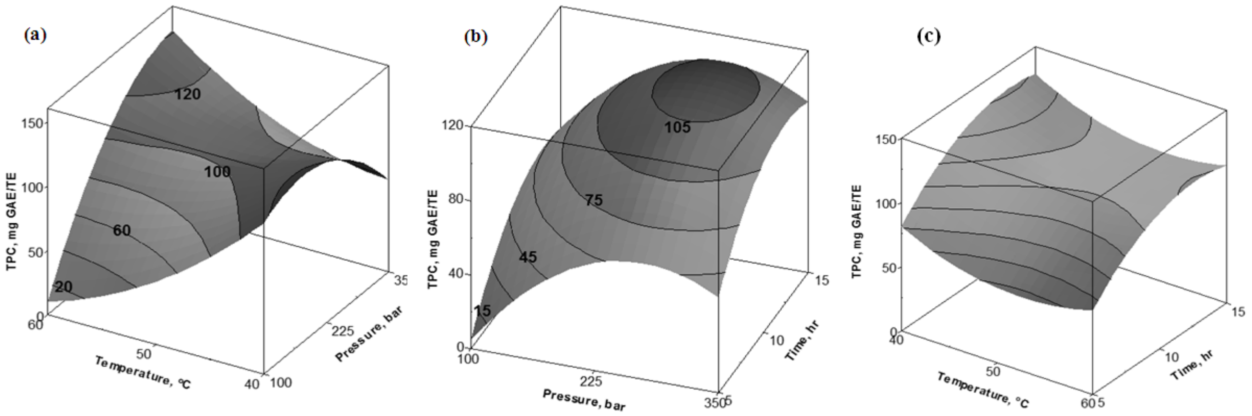

Figure 3. (a) Effect of temperature and pressure on TPC, (b) Effect of pressure and time on TPC, (c) Effect of temperature and time on TPC

The coefficients of the response surface model as provided by Eq. 1 were evaluated. The final predictive equation obtained was as given in Eq. 2 and Eq. 3

$$\begin{aligned}\mathrm{TPC} &= 636.009 - 0.6986X_1 - 23.503X_2 \\ &+22.395X_3 - 0.0019X_1^2 + 0.4191X_2^2 \\ &-0.7814X_3^2 + 0.03535X_1X_2 \\ &-0.0034X_1X_3 - 0.030X_2X_3\end{aligned} \tag{2}$$

$$\begin{aligned}Yield &= 10.445 - 1.895X_1 - 1.356X_2 + 1.066X_3 \\ &-0.472X_1^2 + 0.935X_2^2 - 0.371X_3^2 + 3.920X_1X_2 \\ &+0.078X_1X_3 - 0.767X_2X_3\end{aligned} \tag{3}$$

The effect of pressure, temperature and extraction time on yield are also shown in response plots (Figure 4a-b). In the present study the effect of linear terms is not significant as $p > 0.05$, but there is significant effect of interaction between pressure and temperature which increases the yield and total phenolic content ($p < 0.05$). The highest extraction yield of extract and total phenolic content obtained at 350 bar, 60°C and 15 hr was 2.83% and 154 mg GAE/tot extract which was well matched with the predicted yield. At lower pressure the yield of phenolic content is less due to the solubility of phenolic acid are less at the less density of CO2. At higher pressure yield increases significantly.

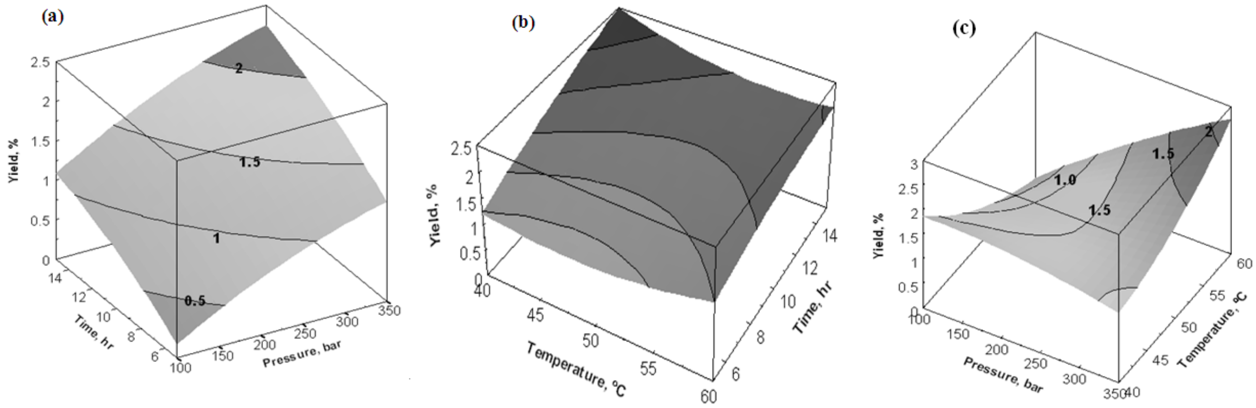

Figure 4. (a) Effect of pressure and time on yield of extract, (b) Effect of temperature and time on yield of extract, (c) Effect of temperature and pressure on yield of extract

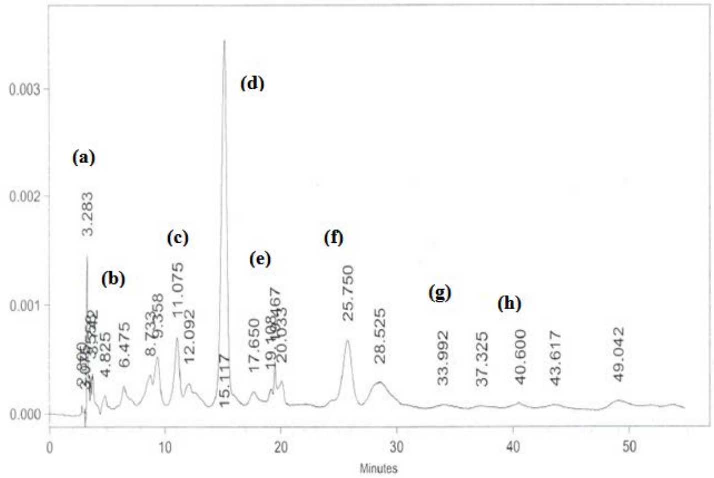

Figure 5. HPLC chromatogram of SC-CO_2 extract of Mango ginger at optimum conditions
(a) Gallic acid, (b) Protocatuchic acid, (c) Gentisic acid, (d) Caffeic acid, (e) Syringic acid, (f) p-Coumaric acid, (g) Ferulic acid, (h) Cinnamic acid

3.3. HPLC Analysis

High performance liquid chromatography has been done for the qualitative analysis of the extract for phenolic acids at highest extraction yield. The compounds are identified on the basis of retention time compared with the standard chromatogram of the pure phenolic acids. The chromatogram is shown in Figure 5.

4. Conclusion

In the present study Supercritical carbon dioxide extraction process was optimized for the extraction of phenolic compounds from mango ginger powder using three levels of Box-Bhenken design from RSM. The high correlation of the mathematical model indicated that a quadratic polynomial model could be employed to optimize the phenolic acids yield by supercritical carbon dioxide extraction. At high pressure, the influence of the temperature on solubility is predominated by the solid vapor pressure effect more than the CO2 density variations. The optimum conditions were found to be 350 bar, 60°C and 15 hrs. Under the optimum conditions, total phenolic content obtained was 154 mg GAE/total extract, which was well matched with the predicted yield.

References

[1] Policegoudra, R.S., Aradya, S.M., “Biochemical changes and antioxidant activity of mango ginger (Curcuma amadaRoxb.) rhizomes during postharvest storage at different temperatures”. *Postharvest Biology and Technology*, vol. 46 no. 2, pp. 189-194, 2007.

[2] Gholap, S.A., Bandyopadhyaya, C., “Characterization of mango-like aroma in *Curcuma amada* Roxb”. *Journal of Agricultural and Food Chemistry*, vol. 32, no. 1, pp. 57-59, 1984.

[3] CSIR (Council of Scientific and Industrial Research), *Wealth of India – raw materials*. New Delhi, vol. 2, pp. 401, 1950.

[4] Policegoudra, R.S., “Functional properties of bioactive molecules from mango ginger (Curcuma amadaRoxb.) and its applications in food”. PhD Thesis, Universityof Mysore, India, 2008.

[5] Manohar, B., Uday Shankar, K., “Extraction Modelling and characterization of bioactive components from Psoralea corylifolia L. Obtained by supercritical carbon dioxide”. *Journal of Food Process Technology*, vol. 3, no. 2, pp. 144, 2012.

[6] Pereira, C.G., Meireles, M.A.M., “Supercritical Fluid Extraction of Bioactive compounds: Fundamentals, Application and Economic Perspectives”. *Food and Bioprocess Technology*, vol. 3, no. 3, pp. 340-372, 2010.

[7] Khajeh, M., “Optimization of process variables for essential oil components from satureja hortensis by supercritical fluid extraction using box behnken experimental design”. *Journal of supercritical Fluids*, vol. 55, no. 3, pp. 944-948, 2011.

[8] Jai, D., Li, S., Xiao, L., “Supercritical CO_2 extraction of plumula nelumbinis oil: Experimental and modeling”. *Journal of Supercritical Fluids*, vol. 50, no. 3, pp. 229-234, 2009.

[9] Cheah, E. L. C., Chan, C. L., Heng, P. W. S., “Supercritical Carbon dioxide and its application in the extraction of active principles from plant material”. *Asian Journal of Pharmaceutical Sciences*, vol. 1, no. 1, pp. 60, 2006.

[10] Ixtaina, V. Y., Vega, A., Nolasco, S. M., Tomas, M. C., Gimeno, M., Barzana, E., Tecante, A., “Supercritical carbon carbon dioxide extraction of oil from Mexican chia seed (salvia hispanica L.). Characterization and process optimization”, *Journal of Supercritical Fluids* vol. 55, no. 1, pp. 192-199, 2010.

[11] Meure, L.A., Foster, N.R., Dehghani, F., “Conventional and Dense Gas Techniques for the Production of Liposomes: A Review”, AAPS Pharmscitechology, vol. 9, no. 3, pp. 798-809, 2008.

[12] Herrero, M., Mendiola, J.A., Cifuentes, A., Ibáñez, E., “Supercritical fluid extraction: Recent advances and applications”. *Journal of Chromatography A*, vol. 1217, no. 16, pp. 2495-2511 16 2010.

[13] Zarena, A.S., “Characterization of bioactive compounds from *Garcinia mangostana* L. obtained by supercritical fluid carbon dioxide process”. PhD thesis, University of Mysore, India, (2011).

[14] Manohar, B., Udaya Sankar, K., “Enrichment of bakuchiol in supercritical carbon dioxide extracts of chiba seed (*Psoralea corylifolia* L.) using molecular distillation-Response surface methodology”. *Biotechnology and Bioprocess Engineering*, vol. 14, no. 1, pp. 112-117, 2009.

[15] Bas, D., Boyac, I.H., “Modeling and optimization I: Usability of response surface methodology”. *Journal of Food Engineering*, vol. 78, no. 3, pp. 836-845, 2007.

[16] Singleton, V.L., Rossi, Jr. J.A., “Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents”. *American Journal of Enology and Viticulture*, vol. 16, no. 3, pp. 144-158, 1965.

[17] Siddaraju, M.N., Dharmesh, S.M., “Inhibition of Gastric H+, K+-ATPase and Helicobacter pylori Growth by Phenolic Antioxidants of Curcuma amada”. *Journal of Agricultural and Food Chemistry*, vol. 55, no. 18, pp. 7377-7386, 2007.

[18] Zarena, A. S., Manohar, B., Udaya Sankar, K., “Optimization of Supercritical Carbon Dioxide Extraction of Xanthones from Mangosteen Pericarp by Response Surface Methodology”. *Food and Bioprocess Technology*, vol. 5, no. 4, pp. 1181-1188, 2012.

[19] Kong, Y., Fu, Y-J., Zu, Y-G., Liu, W., Wang, W., Hua, X., Yang, M., “Ethanol modified supercritical fluid extraction and antioxidant activity of cajaninstilbene acid and pinostrobin from pigeonpea [Cajanus cajan (L.) Millsp.] leaves”. *Journal of Food Chemistry*, vol. 117, no. 1, 152-159, 2009.