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Quantification of Curcumin Obtained from Supercritical Carbon Dioxide Extraction of *Curcuma domestica* Valet. Rhizomes

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ABSTRACT

Background: Curcumin is well known bioactive compound available in *Curcuma domestica* Valet *i.e.* turmeric. The research work was carried with intention to get maximum yield of medicinally active constituents (Curcumin) of *Curcuma domestica* Valet. rhizomes through supercritical fluid extraction.

Methods: The experiments were conducted in the year 2013-14. Process parameters namely pressure, temperature, flow rate and particle size were optimized using a Taguchi method. During investigation, L9 orthogonal array experimental design was followed.

Result: The optimized values of pressure, temperature, flow rate and particle size obtained were 35 MPa, 40°C, 25 g/min and 0.246 mm, respectively. Significant improvement in the yield of curcumin and extract was obtained under optimum operating conditions.

Key words: Curcumin, Optimization, Supercritical fluid extraction, Taguchi method.

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INTRODUCTION

Natural products have been our single most successful source of medicines (Shinde and Dhalwal, 2007). Recent years have shown a growing popularity and faith in herbal based medicines worldwide. *Curcuma domestica* Valet. is commonly known as turmeric which belongs to the Zingiberaceae family. The essential oil of turmeric rhizome has some important components such as curcuminoids, ar-turmerone and turmerone which were studied in detail by number of workers (Gopalan *et al.*, 2000 and Silva *et al.*, 2005). The yellow colour and biological activities of turmeric is mainly due to the Curcuminoids (Jayaprakasha *et al.*, 2002). The Curcuminoids consist of a mixture of three constituents namely, curcumin [1,7-bis (4-hydroxy-3-methoxyfenil)-1,6-heptadiene-3,5-dione], demethoxy-curcumin (DMC) [1-(4-hydroxy-phenyl)-7-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] and bisdemethoxycurcumin (BDMC) [1, 7 – bis (4-hydroxyphenyl) -1, 6-heptadiene-3, 5-dione] (*Manzan et al.*, 2003; Peret-Almeida *et al.*, 2005).

The medicinally active component of curcuminoids is curcumin, which is about 50-60% of total curcuminoids by weight. It has potential to heal liver disorders, digestive disorders, skin diseases, wound, atherosclerosis, bacterial infection and eye disorder. Leal *et al.* (2003) have been confirmed that the curcumin have anti-microbacterial activity as well as selective anticancer activities. Braga *et al.* (2003) have found it blood purifier and anti-oxidative. According to Lantz *et al.* (2005) and Kao *et al.* (2007), curcumin is anti-mutagenic and anti-inflammatory and anti-fungicidal activities.

Extraction involves the separation of medicinally active constituents from the inactive or inert part of plant and herb by the use of selective solvents. Conventional methods of extraction are maceration, percolation and soxhlet extraction. These techniques have suffered from number of drawbacks such as use of organic solvents, high time consumption and labour intensiveness. Low yield potential and poor quality are also the limitations of the conventional methods. Nowadays these extraction techniques are dominated by advanced techniques like Supercritical Fluid Extraction (SFE), ultra-sound assisted extraction, *etc.* In SFE, properties of fluids under supercritical condition are exploited to maximize the extraction of plant constituents and minimize the degradation of thermolabile component. The advantages of using SFE are high repeatability, accuracy and selectivity (Krithika *et al.*, 2015). Supercritical carbon dioxide is used as a solvent used in SFE which is nontoxic, inert, provides clear extracts and has shorter extraction time. The process does not leave any organic residue or leave very little residue (in case of co-solvent) than conventional extraction methods (Shinde and Dhalwal, 2007).

Thus, the study was aimed to optimize the extraction conditions for supercritical fluid extraction of curcumin from *Curcuma domestica* Valet.

MATERIALS AND METHODS

Chemicals and materials

Dried rhizomes of *Curcuma domestica* Valet. (Turmeric) were obtained from local market. The methanol (≥ 99%), ethanol (≥ 99%) and acetone (≥ 99%) were obtained from Merck (Mumbai, India). Carbon dioxide (99.9%) was provided by Kentech Associate Pvt. Ltd. (Kanpur, India). Pure curcuminoids standard (99% w/w) was obtained from Centre for Cellular and Molecular Biology (Hyderabad, India).

The experiments were conducted in the year 2013-14 at Industrial Pollution Control Lab, Department of Chemical Engineering and Technology, IIT (BHU), Varanasi. The dried rhizomes were ground by edge runner mill (Elico, Hyderabad, India) and average particle size were determined using sieve shaker fitted with 0.833 mm, 0.417 mm and 0.246 mm sieves. After particle size analysis, the different sized materials were packed in plastic bags and stored in refrigerator for further investigation.

Taguchi methodology

The Taguchi method is a commonly adopted approach for optimizing operating parameters. The method was originally proposed as a means of improving the quality of products through the application of statistical and engineering concepts. The effects of several process parameters can be determined effectively by carrying out matrix experiments based on the Taguchi's orthogonal design. Since experimental procedures are expensive and time consuming, the need to satisfy the design objectives with the least number of tests is clearly an important requirement. The Taguchi method involves laying out the experimental conditions using specially constructed tables known as "orthogonal arrays". The use of these tables ensures that the experimental design is both straight forward and consistent. Adopting the Taguchi approach, the number of analytical explorations required to develop a robust design are significantly reduced which resulted in minimization of overall testing time and the experimental costs (Roy, 2001). Orthogonal experiment selects the parameters affecting experimental target as factors. Each factor has several levels. Then orthogonal table was selected by the number of factors and levels.

Temperature and pressure are two most important parameters in the supercritical fluid extraction. The increase of temperature reduces the density of SC-CO_2 (for a fixed pressure) thus reducing the solvating power of the supercritical solvent; but it also increases the vapour pressure of the compounds to be extracted which increases the tendency of these compounds to pass in the fluid phase. Therefore, the extraction pressure is most relevant parameter and can be used to tune the selectivity of the supercritical fluid. However, the general rule is that the higher is the pressure, the larger is the solvent power and the smaller is the extraction selectivity. The other crucial parameters are CO_2 flow rate, particle size and duration of the process (extraction time).

In the present study, a L9 orthogonal table was used which formed with four factors (pressure, temperature, flow rate of CO_2 and particle size) and three levels each (Table 1). The design completes the optimization process in just 9 runs as against 81 trials if a full factorial design was followed. However, each combination was performed in three replications to minimize the experimental error. Though L9 orthogonal array has few experimental conditions, number of replication employed overcomes the higher orthogonal arrays like L27. The initial parameters were selected on the basis of considering general rule as shown in Table 2. After conducting the experiments, the results were converted into signal-to-noise (S/N) ratio data. The Signal-to-Noise (S/N) ratio was computed for each level of process parameters. Regardless of the category of the quality, the lower is better, the higher is better and the nominal is better, a larger S/N ratio corresponds to better quality characteristics. In other words, the optimal level of the process parameters is the level with the greatest S/N ratio. This was the foundation for the decision of the optimum level for each factor. Since the current study takes the percentage extraction of the quality characteristics and the main objective of the study was higher yield for the extract and curcumin quantity, hence, the higher is better criterion was applied when evaluating the S/N ratios of the various extraction parameters. S/N ratio for the higher is better is:

$$\text{S/N ratio} = -10 \log \frac{1}{n} \sum_{i=1}^{n} \frac{1}{Y_i^2}$$

Where,

Y_i is Quality of i^{th} trial, n is number of trials.

Supercritical fluid extraction

The supercritical fluid extraction system and components were acquired from Thar Technologies, Inc. (Pittsburgh, PA) which had the following main parts: extraction vessel of 500 mL capacity, model P-series high pressure dual piston pump, electrical heater, automated back pressure regulator model BPR-A-200B and collection vessel of 500 mL capacity. The

Table 1: Taguchi L9 orthogonal array.

Experiment no.	A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Note: A, B, C and D are factors and 1, 2 and 3 are levels.

Table 2: Extraction parameters.

Factors	Levels		
	1	2	3
Temperature, °C, (A)	35	40	45
Pressure, MPa, (B)	25	30	35
Flow rate, g/min, (C)	15	20	25
Average particle size, mm, (D)	0.833	0.417	0.246

system was controlled by software namely "*Process suite program*" provided by Thar Technologies, Inc. (Pittsburgh, PA).

For each experimental run, 50 g of turmeric powder of uniform size was taken and loaded into extraction vessel. Liquid CO_2 was supplied from a cylinder with siphon tube arrangement. Before compression, the CO_2 was cooled by passing it through a chiller. Cooled CO_2 was pumped to the extraction vessel using high pressure pump through an electric heater where CO_2 was heated upto required extraction temperature. The CO_2 at supercritical state flowed into the extractor, where it is compressed upto required pressure and came in contact with the turmeric powder. Pressure was controlled by automated back pressure regulator (ABPR). After extraction the oil-laden CO_2 was led into a collection vessel and finally released to the atmosphere. Extract of turmeric was collected from bleed valve of collection vessel into a sample bottle covered with silver foil and was kept in refrigerator. The extraction time was 180 min. Table 3 shows the results of the corresponding experiment.

Solvent extraction

50 g of turmeric powder of known size was taken along with 400 mL of methanol for the extraction in a soxhlet extraction apparatus of 1000 mL capacity (Kulkarni *et al.*, 2012). Heating mantle was used to heat the solvent. Water was circulated as a cooling medium through the condenser. The temperature of heater was maintained at 70°C throughout the experiment. Evaporation process was completed in 120 minutes. Extract was stored in sample bottle covered with silver foil and was kept in refrigerator for further analysis.

Table 3: Experimental Results of Supercritical CO_2 Extraction for 180 minute extraction.

Exp. no.	Extraction yield* (%, w/w)	S/N ratio	Curcumin (mg/50gm)	Curcumin (mg/g)	S/N ratio
1	2.55	8.13	2.18	0.04	6.77
2	3.26	10.26	11.27	0.23	21.04
3	4.47	13.01	19.04	0.38	25.59
4	4.11	12.28	18.03	0.36	25.12
5	4.41	12.89	19.44	0.39	25.77
6	3.37	10.55	11.64	0.23	21.32
7	3.47	10.81	12.29	0.25	21.79
8	3.55	11.00	8.76	0.18	18.85
9	3.46	10.78	12.03	0.24	21.61

Note: *All values are average of three replications.

Quantification of curcumin

Quantification of curcumin was done by UV-Visible Spectrophotometer (159 SL, Elico, Hyderabad, India). For curcumin, maximum absorbance was obtained at 427 nm wavelength. Standards of different concentration were prepared by diluting the curcumin using ethanol as a solvent. Calibration curve between concentration and absorbance was obtained and found straight line as given below.

$$A = 0.173\ C - 0.008$$

Where,

A is absorbance and C is concentration in mg/L.

Concentration of curcumin in each sample was calculated by above equation.

RESULTS AND DISCUSSION

The ANOVA calculated for the results revealed that temperature, pressure and particle size had significant effect on the extraction yield as well as the concentration of curcumin ($p<0.5$). However, the flow rate had no significant effect on the extraction yield and thus on the concentration of curcumin (Table 4).

Effect of temperature

Fig 1 shows that with increase in temperature, quantity of extract was increased for temperature rise from 35 to 40°C and then decreasing by further increase in temperature to 45°C. This was due to two counter effects playing role with increase in temperature in the SFE. There was increase in the quantity of extract when temperature rose because of the increase in the vapour pressure of the oil. Simultaneously rise in temperature is also responsible for decrease in density of supercritical fluid hence decrease in the dissolving capacity which resulted in decrease in the quantity of extract (Said *et al.*, 2014). In the temperature range of 35 to 40°C the first effect dominated and above 40°C the second effect dominated. Fig 2 also shows that the curcumin quantity is higher at 40°C. Graphical representation of the analysis of means (Fig 1 and 2) indicates that 40°C is optimum temperature to obtain maximum quantity of extract.

Effect of pressure

Fig 1 and 2 show that when pressure was increased from

Table 4: ANOVA for result obtained during extraction of curcumin using L9 orthogonal array design ($p<0.5$).

Parameters	Degree of freedom	Sequentialsums of squares	Adjusted sums of squares	Adjusted mean squares	F	P value
Temperature	2	0.84	0.84	0.28	1.48	0.02
Extraction pressure	2	1.86	1.86	0.62	22.70	0.39
CO_2 Flow rate	2	0.08	0.08	0.03	1.48	0.54
Particle size	2	3.87	3.87	1.36	15.32	0.44
Residual error	7	1.06	1.06	0.35		
Total	15	7.71				

25 to 30 MPa and then from 30 to 35 MPa, the quantity of extract is increasing continuously. This is attributed to the increase in density of CO_2 with increase in pressure. Higher density favours the better yield of extract in supercritical fluid extraction. Graphical representation (Fig 1 and 2) indicates that 35 MPa pressure was optimum pressure to obtain maximum yield of extract and quantity of curcumin. Al-Rawi *et al.* (2013) were also observed that increase of pressure from 20.7 to 41.4 MPa increased the extraction yield from 32.2 to 36 g of nutmeg oil per 100 g seed.

Effect of CO_2 flow rate

From Fig 1 and 2, it was observed that the flow rate was increased from 15 g/min to 20 g/min and further to 25 g/min, quantity of extract and curcumin increases. This is because increase in CO_2 flow rate resulted in more availability of CO_2 in the extractor which in turn increased the inter-molecular interaction between CO_2 and the solute, thus enhanced solute dissolution. Although the ANOVA showed that the supercritical carbon dioxide had no significant effect on the yield. Graphical representation of the analysis of means (Fig 1 and 2) indicates that 25 g/min flow rate of CO_2 was optimum to obtain maximum yield. The similar behaviour was observed in extraction of bioactive compound from soybean (Jokic *et al.*, 2012) and ginger (Said *et al.*, 2014).

Effect of particle size

The effect of particle size on overall extraction yield and quantity of curcumin are shown in Fig 1 and 2. It was observed that for decrease in particle size from 0.833 to 0.417 mm and further to 0.246 mm, the extraction yield and quantity of curcumin increases. This is because of reduction in the size raw material resulted in enhanced surface area, disrupted cell walls and also increased the ratio of broken cells to intact cells and thus reducing the mass transfer

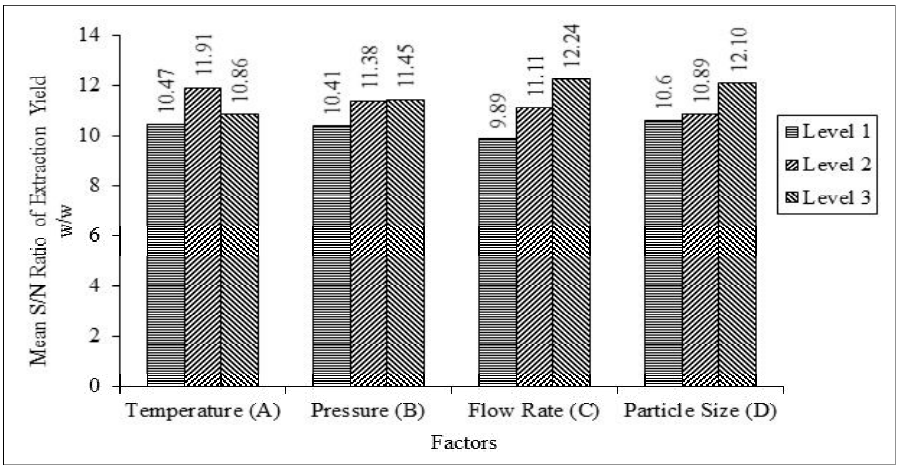

Fig 1: Response graph of the variation of the mean S/N ratios of percentage extraction plotted against the four extraction parameters for 180 minutes of extraction#

#Levels of each independent variable (factors) were as shown in Table 2.

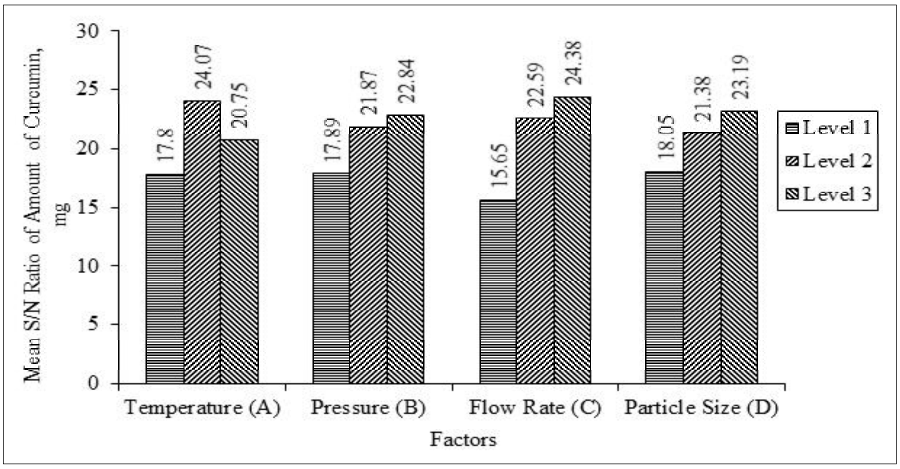

Fig 2: Response graph of the variation of the mean S/N ratios of curcumin quantity plotted against the four extraction parameters for 180 minutes of extraction#

#Levels of each independent variable (factors) were as shown in Table 2.

resistance. Due to decrease in mass transfer resistance, the essential oil was more accessible to the solvent and consequently, the extraction yield increased (Gopalan *et al.*, 2000). The reduction in extraction yield with increasing particle size indicated that oil was not transported through the unbroken cell walls and only surface oil was removed (Said *et al.*, 2014). Graphical representation of the analysis of means (Fig 1 and 2) indicates that 0.246 mm average particle size was optimum to obtain maximum yield of extract and quantity of curcumin.

Soxhlet extraction

Solvent extraction was performed by using methanol as solvent. The average particle size of 0.246 mm was taken for the experiment. The yield of extract and quantity of curcumin obtained by using methanol were 4.8% and 0.34 mg per gram of raw material respectively.

Yield of extract and curcumin under optimum operating conditions

As discussed in the previous sections, the optimized values of pressure, temperature, flow rate and particle size obtained using Taguchi method were 35 MPa, 40^{o}C, 25 g/min and 0.246 mm respectively. Under optimum operating conditions 5.63% extraction and 0.48 mg of curcumin per gram of raw material was obtained. The optimized yield of curcumin was 23.7% more than best result obtained under non optimized conditions in supercritical fluid extraction (Table 3, 19.44 mg per 50 g of raw material or 0.388 mg of curcumin per gram of raw material) and 41.2% more than that by soxhlet extraction.

CONCLUSION

In the supercritical fluid extraction of curcumin using CO_2 as solvent, the optimum operating conditions were obtained using Taguchi experimental design method. Under optimum operating conditions, yield of curcumin and extract were improved significantly over the unoptimized supercritical and soxhlet extraction results. The results illustrate that the Taguchi method can be effectively used to optimize the operating parameters for better yield.

Conflict for interest: None.

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