Optimization of Curcumin Extraction from Turmeric Powder using a Box-Behnken Design (BBD)

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Turmeric powder, obtained by grinding rhizomes of Curcuma longa, is a valuable source of curcumin, which behind its colouring properties, presents antioxidant, anti-inflammatory, antimicrobial properties and could be used in treating Alzheimer or cancer. Optimization of solvent extraction of curcumin from turmeric powder, using a Box-Behnken Design was performed, studying the influence of temperature, liquid/solid ratio and extraction time on the relative extraction efficiency.

Keywords: curcumin, solvent extraction, optimization, Box-Behnken Design

The yellow and flavourful powder called as "Turmeric" is provided by grinding the dry rhizomes of *Curcuma longa* L., a tropical herb native to southern Asia. It is used especially as a food additive, colouring and flavouring agent, being a common spice in the Asian cuisine, but also in household medicine in Southern Asia. The largest turmeric producer and exporter in the world is India [1-3].

In recent years, researches proved the medicinal value of turmeric. The active compounds of turmeric are the curcuminoids (curcumin and two other related compounds, viz. demethoxy curcumin and bis-(demethoxy) curcumin) and ar-turmerone, the major component of the essential oil. Anti-inflammatory, antibacterial, antifungal, antiparasitic and antimutagenic activities of curcuminoids have been reported in the literature [4-7]. Also, some researchers reported the use of curcumin in treating diseases such as Alzheimer or cancer [1, 8]. The quality of oil depends on the applied extraction procedure [6]. Solvent extraction, hot pressurized water extraction and supercritical carbon dioxide extraction have been demonstrated to be feasible for recovering fat-affinitive compounds from natural materials [9]. Soxhlet extraction and other energy consuming conventional methods, such as steam distillation, were for a long time the main procedures for turmeric oil separation from the dry rhizomes of *Curcuma longa*. Recently new procedures were tested in curcumin extraction, such as enzyme assisted extraction, microwave or ultrasound assisted extraction [5, 10, 11]. Extractions were performed with acetone, methanol, ethanol, dichloromethane or hexane as solvents [7, 12]. An optimized batch solvent extraction could be considered as a feasible method for curcumin extraction. Optimization of an extraction process could be achieved by studying the influence of each factor separately. This procedure needs a laborious experimental effort. However, through the application a statistical method for optimising the extraction process the number of experiments required could be minimized [12-15]. Among the most common designs, Box–Behnken design (BBD) has been widely used in optimization of many chemical and physical processes [16].

The aim of this paper is to investigate the curcumin extraction from turmeric powder and to optimize the extraction using a Box-Behnken Design (BBD). The influence of extraction conditions such as temperature, liquid/solid ratio and extraction time on the curcumin relative extraction efficiency was studied.

Experimental part

Materials and chemicals

Turmeric powder was purchased from a local market. Ethanol (96% purity) and n-hexane (analytical grade) were used as solvent in extraction. Curcumin (purity 98% by HPLC) was used for calibration curve in UV-Vis spectroscopic analysis of the extracts. All reagents were purchased from SIGMA ALDRICH and used without further purification.

Extraction procedure

Soxhlet extraction was used in order to determine the maximum curcumin content in the oleoresin of the raw material. In conventional Soxhlet apparatus, 2g of turmeric powder were accurately weighed and extracted with ethanol for 24 h. The curcumin content of the extract was determined using UV-Vis spectroscopy. Batch extraction was carried out for all extraction experiments. The stirring speed was set at the appropriate level, in order to ensure the suspension of solid in the liquid phase and a good contact between them. In a closed flask 2 g of turmeric powder was carefully weighed and contacted under stirring with different volumes of ethanol and n-hexane. Extractions were performed in the temperature range of 25 – 60 °C.

All experiments were conducted in order to find out the extraction conditions which correspond to a maximum curcumin content of the extracts. Relative extraction efficiency, Y, defined as follows, was used in process evaluation:

$$Y = \frac{mass\ of\ extracted\ curcumin\ /\ turmeric\ powder\ (w/w)}{mass\ of\ extracted\ curcumin\ obtained\ in\ Soxhlet\ extraction/\ turmeric\ powder\ (w/w)} \quad (1)$$

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Independent variables	Symbol coded	Range and levels		
		-1	0	1
Temperature (°C)	X_1	25	40	55
Liquid/solid ratio (mL/g)	X_2	20	30	40
Extraction time (h)	X_3	1	2.5	4

Table 1
EXPERIMENTAL RANGE, LEVEL AND CODE OF INDEPENDENT VARIABLES FOR CURCUMIN EXTRACTION FROM TURMERIC POWDER

All experiments were made in triplicate.

Analysis of the extracts

Turmeric oleoresins commonly contain 30–45% curcuminoid pigments. Consequently, the curcumin content in the extracts was used to evaluate the extraction efficiency. Curcumin was determined by measuring the absorbance of an ethanol curcumin solution, using an UV/Vis Cintra 6 Spectrophotometer (GBS Scientific, Australia), at fixed wavelength at 425 nm.

Experimental design

The extraction conditions of curcumin from turmeric powder were optimized using a Box-Behnken Design (BBD). Among many factors which are known to influence extraction yield three variables were chosen: temperature, extraction time and the ratio between solvent/ turmeric powder. Therefore the process independent variables were: temperature (X1), liquid/solid ratio (X2) and extraction time (X3). From preliminary experiments the range for each independent variable was selected. Each parameter was tested at three levels coded as (–1) for lower level, (+1) for higher level, and a central coded value considered as zero (0) as it is depicted in table 1. The result was an experimental design with 17 experimental points, including five central points. All the experiments were carried out in a randomized order to minimize the effects of unexpected variability in the observed response. The chosen response was the relative extraction yield (Y1).The statistical package software STATISTICA trial version 10.0 (Stat Soft Inc., Tulsa, USA) was used for experimental design analysis and data processing. The regression model is a polynomial second-order equation which contains a constant term (β_0), coefficients of linear terms (β_1, β_2, and β_3), coefficients of quadratic terms (β_{11}, β_{22}, and β_{33}) and coefficients of cross product terms (β_{11}, β_{13}, and β_{23}).

$$Y_1 = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \\ + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

where Y_1 is the response (relative extraction yield).

Results and discussions

In Soxhlet extraction, using ethanol as solvent, a maximum of 4.1% curcumin content in the extract was obtained. The performances of the two solvents tested in batch extraction are presented in figure 1.

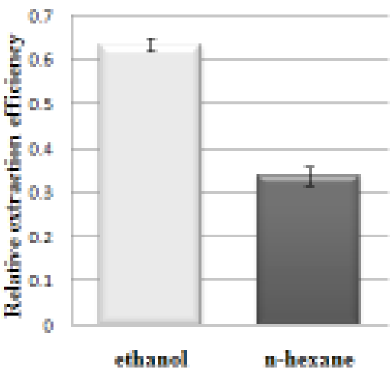

Fig.1.Influence of the solvent used in curcumin extraction on the relative extraction efficiency

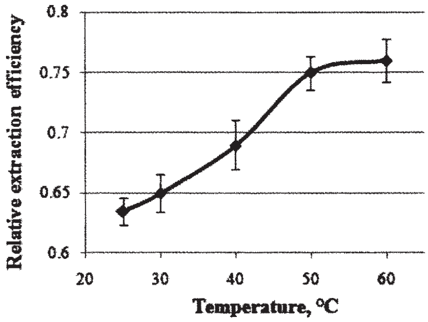

Fig.2. Relative extraction efficiency vs. extraction temperature (liquid/solid ratio = 20/L, extraction time 4 h)

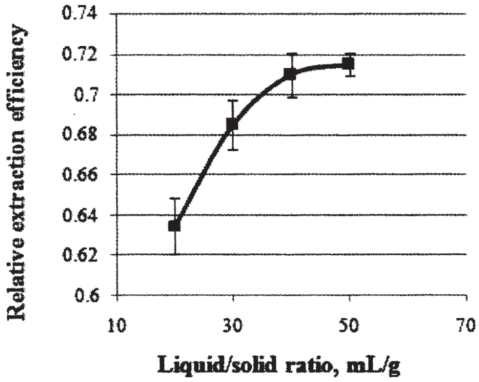

Fig.3. Relative extraction efficiency vs. liquid/solid ratio (extraction temperature 25°C, extraction time 4 h)

One can see that ethanol is more efficient as n-hexane, consequently it was used in all other experiments. The effect of the extraction temperature and liquid/solid ratio on the relative extraction efficiency is presented in figure 2 and 3.

It could be seen that the relative extraction efficiency increases with temperature, but an increase over 50 °C brings only a slight rising in the extraction efficiency. Also, increasing the liquid/solid ratio over 40/1 mL/g has conducted only to a minor increase of the relative extraction efficiency. All these preliminary experiments were used to select the range of variation of the influencing parameters in order to find the optimum conditions for maximum curcumin content of the extracts. Box-Behnken design matrix of independent variables in coded units along with experimental and predicted values for curcumin relative extraction yield is presented in table 2.

A second order quadratic model which predicts the relative extraction yield was obtained by applying multiple regression analysis on the experimental data and it is represented by the following equation in term of coded factors:

$$Y_1 = 0.8094 + 0.073X_1 + 0.0232X_2 + 0.0292X_3 - \\ - 0.0598X_1^2 - 0.0383X_2^2 - 0.0683X_3^2 + \\ 0.0162X_1X_2 + 0.0312X_1X_3 - 0.00775X_2X_3 \quad (3)$$

Student's t-test was used for checking the statistical significance of the regression coefficient. The adequacy of the model was determined by evaluating the coefficient of determination (R^2) and the F-test value was obtained from the analysis of variance (ANOVA) presented in table 3. Significance of the model terms is checked by their

Table 2

BOX-BEHNKEN DESIGN MATRIX OF INDEPENDENT VARIABLES IN CODED UNITS ALONG WITH EXPERIMENTAL AND PREDICTED VALUES FOR CURCUMIN RELATIVE EXTRACTION YIELD

Run	Coded variable levels			Yield of curcumin	
	X_1	X_2	X_3	Exp. values*	Predicted values
1	-1	-1	0	0.635	0.646
2	1	-1	0	0.760	0.759
3	-1	1	0	0.630	0.630
4	1	1	0	0.820	0.808
5	-1	0	-1	0.610	0.610
6	1	0	-1	0.680	0.693
7	-1	0	1	0.620	0.606
8	1	0	1	0.815	0.814
9	0	-1	-1	0.670	0.657
10	0	1	-1	0.691	0.689
11	0	-1	1	0.730	0.731
12	0	1	1	0.720	0.732
13	0	0	0	0.812	0.809
14	0	0	0	0.810	0.809
15	0	0	0	0.813	0.809
16	0	0	0	0.800	0.809
17	0	0	0	0.812	0.809

*Values represent the average of the experiments performed in triplicate

respective P values; when these values are less than 0.05 the model terms are significant. In table 3 all the significant terms were enlightened. Analysis of P-values depicted in table 3 show that among the variables tested, the linear and quadratic terms of temperature and time and the quadratic term of solvent/solid ratio had a significant effect on the extraction yield. From the cross terms only the products between temperature and time and between temperature and liquid/solid ratio have a significant effect also upon relative extraction yield.

The determination coefficient has a high value (R^2= 0.9895) which means a good correlation between the experimental values and the predicted ones. The Adj. R^2 was determined as 0.976 which means that only 2.4% variation could not be explained by the model. The model was found to be highly significant having a high F high value 73.27 ($P<0.0001$) from the Fischer's F-test.

The Pareto chart could also be used to identify the significant factors. As it can be seen in figure 4 linear and quadratic terms of temperature, time and solvent/solid ratio and the product between temperature and time have a confidence level greater than 95% and could be considered as significant.

In order to gain a better understanding of the results, the predicted models are presented in figure 5 as the 3-D response surface plots. These plots are obtained depicting two variables within experimental range and keeping the third variable constant at zero level.

From figure 5 it can be seen that the extraction efficiency increases with the rising in temperature and time. A great increase in the resulted extraction efficiency was observed when extraction time was increased from 1 h to 2.5 h and when temperature was increased from 25 to 40°C. The effect of liquid solid ratio upon extraction efficiency is less significant than the extraction time and temperature.

Table 3

ANALYSIS OF VARIANCE (ANOVA) FOR THE REGRESSION MODEL OF CURCUMIN RELATIVE EXTRACTION YIELD FOR BBD

Term	Sum of squares	DF	Mean square	F	P
Model	0.1000	9	0.1000	73.270	0.000001[a]
X_1	0.0420	1	0.0420	277.24	0.000001[a]
X_2	0.0005	1	0.0005	3.59	0.00999
X_3	0.0068	1	0.0068	45.13	0.000273[a]
X_1^2	0.0150	1	0.0150	99.36	0.000022[a]
X_2^2	0.0062	1	0.0062	40.78	0.000372[a]
X_3^2	0.0196	1	0.0196	129.60	0.000009[a]
X_1X_2	0.0010	1	0.0010	6.96	0.033474[a]
X_1X_3	0.0039	1	0.0039	25.75	0.001439[a]
X_2X_3	0.0002	1	0.0002	1.58	0.248533
Error	0.00106	7	0.000152		
Total SS	0.101089	16			

R^2=0.9895 Adj R^2=0. 9759

[a]P<0.05 is considered significant

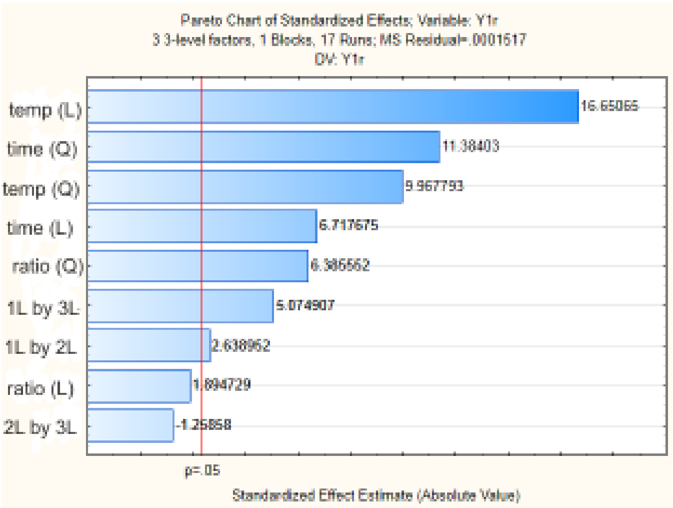

Fig. 4 Pareto chart for the effects of temperature (temp), liquid/solid ratio (ratio), extraction time (time) and of their interactions on the relative extraction efficiency of curcumin from turmeric powder

Optimization of extraction parameters

The aim of this study was the optimization of the extraction conditions in order to obtain a maximum value for relative extraction yield. The optimal values were obtained using Design Expert 8.0.7.1 (trial version, Stat-Ease Inc., Minneapolis, MN, USA) software, by solving the regression equation 3. The coded values for the variables X_1, X_2 and X_3 at optimal point were: X_1=0.51; X_2=0.35 and X_3=0.38.

The optimal value for relative extraction efficiency was found Y_1=0.838. To verify these results, three additional experiments were performed at the following values of the variables: temperature=33°C (instead of 32.65°C obtained from optimization procedure), liquid solid ratio 24 mL/g (instead of 23.6 mL/g obtained from optimization procedure) and extraction time 94 min (instead of 94.2 min obtained from optimization procedure). The relative efficiency of curcumin extraction was determined to be Y_{1exp}=0.830±0.02 in a good agreement with the predicted value.

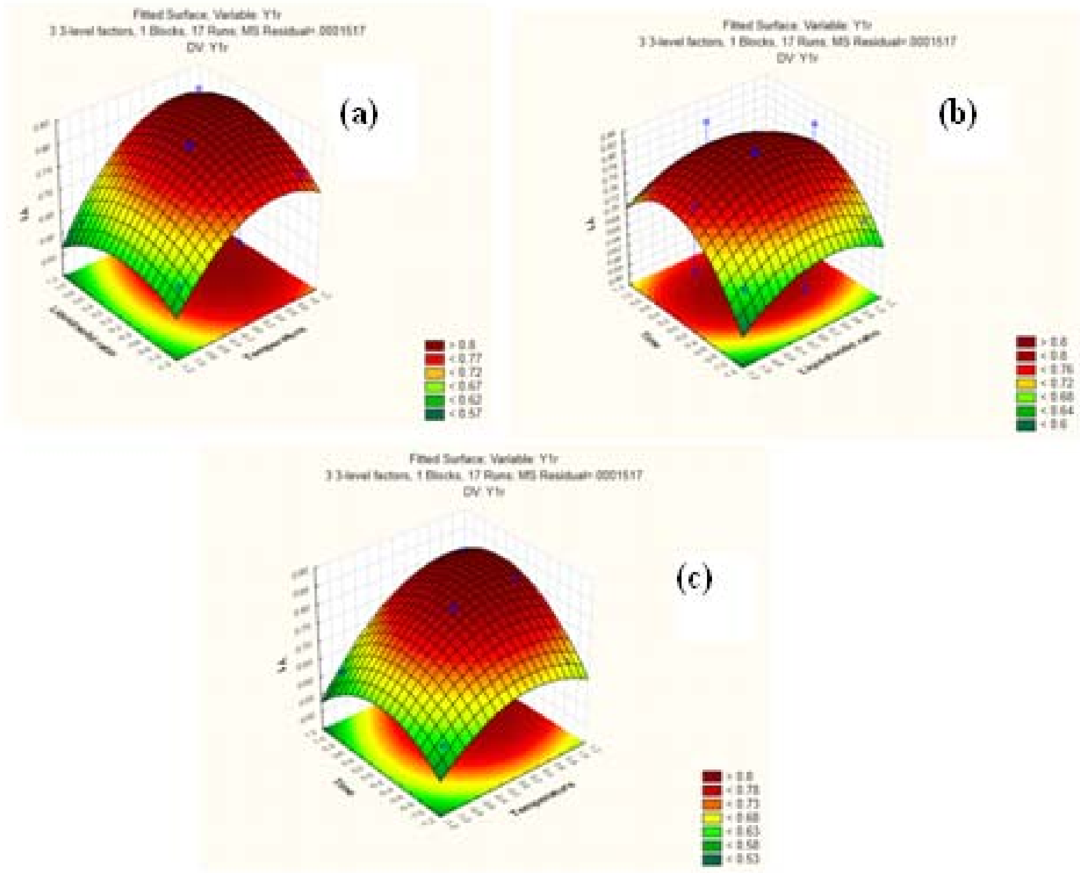

Fig. 5. Response surfaces of curcumin relative extraction efficiency as a function of: (a) temperature and L/S ratio, (b) time and L/S ratio and (c) temperature and time

Conclusions

The aim of this study was to apply Box-Behnken response surface design and numerical optimization in order to underline the influence of the most important process variables upon extraction yield of the curcumin from turmeric powder. The most important factors in the regression equation which influence the relative extraction yield were temperature and time, both as linear and quadratic terms and the quadratic term of solvent/solid ratio. Process optimization was conducted to maximize the relative extraction yield. The experimental value obtained for extraction yield is in a good agreement with that predicted with the quadratic model used for curcumin extraction from turmeric powder.

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