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Optimization of the extraction of curcumin from *Curcuma longa* rhizomes

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Abstract: The aim of this work was to study the effect of dynamic maceration factors upon the curcumin content of *Curcuma longa* L., Zingiberaceae, extracts and to determine the optimum set of parameters for the extraction of curcumin using a 2⁵ full factorial design and the response surface methodology. Under the established conditions, the content of soluble solids and curcumin in the extracts ranged from 0.8 to 3.4%, and from 0.1 to 1.8%, respectively. The most influential variable observed for the extraction was the ethanolic strength of the solvent. The optimized condition involves an extraction time of 12 h, agitation speed of 30 rpm, drug to solvent ratio of 1/6, extraction temperature of 80 °C and the solvent with ethanolic strength of 70%. The data reported herein are useful for further developments of curcuma phytopharmaceutical intermediate products with optimized characteristics.

Introduction

The dried and powdered rhizomes of *Curcuma longa* L., Zingiberaceae, commonly known as turmeric, are used worldwide as a food-coloring agent. Several *in vitro* and *in vivo* studies confirmed that turmeric extracts have powerful biological activities, such as antiinflammatory (Jurenka, 2009), antibacterial (De et al., 2009), antidepressant (Kulkarni et al., 2009), antidiabetic (Wickenberg et al., 2010), antitumor (Wilken et al., 2011), imunomodulatory (Rogers et al., 2010) and gastroprotective (Kim et al., 2005) properties. In addition, it has been successfully used in the treatment of Alzheimer's disease (Ahmed et al., 2010) and cardiac disorders (Morimoto et al., 2010). Owing to its antioxidant properties, turmeric has been widely accepted as one of the spices with the highest antioxidant activity (Wojdyło et al., 2007). The antioxidant activity of turmeric justifies its use in a broad range of applications, including cosmetics (Thornfeldt, 2005), nutraceuticals (Aggarwal, 2010) and phytomedicines (Aggarwal & Harikumar, 2009). These medicinal attributes can be related to turmeric's high content of curcuminoids, especially curcumin, which is considered a chemical marker of this specie (Gupta et al., 2012).

Developing a phytomedicine requires the use of specific processing technologies, including the

extraction of herbal active compounds or chemical markers (Rocha et al., 2008). Several techniques and devices can be applied for this goal. Static (Monedero et al. 1999) and dynamic (Sogi et al., 2010) maceration, percolation (Chaves & Da Costa, 2008), supercritical fluid (Santana et al., 2011), as well as ultrasonic (Costa et al, 2011) and microwave (Mandal et al., 2008) assisted extractions are commonly used. However, due to the specific requirements, such method can be time consuming, require the use of large amount of organic solvent and may have lower extraction efficiencies (Ong, 2004). Moreover, even with the same technique of extraction, for different marker compounds in different plant materials, different operating conditions may be required (Noriega et al., 2012). Thereby, the method and processing conditions employed in the extraction of chemical markers from herbal raw materials play important roles in determining the quality, cost and, overall, the efficacy of the standardized phytopharmaceutical intermediate product (List & Schmidt, 1989).

Based on these considerations, it is of great interest to undertake studies in order to investigate the relationship between extraction parameters and extract properties on the development of turmeric's phytomedicines. The aim of this work was to study the effect of dynamic maceration factors, namely agitation speed, time, drug to solvent weight ratio, extraction temperature and ethanolic strength on the curcumin

and soluble solid contents for *C. longa* rhizomes. Besides, another goal was to determine the optimum set of conditions for the extraction of curcumin using the Response Surface Methodology (RSM).

Material and Methods

Reagents and chemicals

Curcumin (96%) was purchased from Sigma-Aldrich® (Sigma-Aldrich Co., Steinheim, Germany). Acetonitrile was of HPLC grade (Merck KGaA, Darmstadt, Germany). Additionally, citric acid (Merck KGaA, Darmstadt, Germany), ethanol (Chemis Ltda., São Paulo, SP, Brazil) and ultrapure water from a Milli-Q system (Millipore®, Bedford, MA, USA) were used. All other chemicals were of reagent grade and were used without further purification.

Herbal material

The dried *Curcuma longa* L., Zingiberaceae, rhizomes were purchased from the YOD do Brasil Ltda (São Paulo, SP, Brazil), pharmacognostically characterized by Prof. Cid Aimbiré M. Santos and a sample was deposited at the “Herboteca Carlos Stelfled” from the Pharmacognosy Laboratory of the Departamento de Farmácia Universidade Federal do Paraná, with Registry number 122-A. The rhizomes were grounded in a knives mill TE-625 (Tecnal Ltda, Piracicaba, SP, Brazil). Powdered material was stored sheltered from light and moisture for subsequent characterization and use in the extraction studies. The powder moisture content, total ash content, swelling index and size distribution were determined according to the methodologies described in Farmacopéia Brasileira (2010). The results were expressed as mean±SD of three replicates.

Design of experiments, Response Surface Methodology (RSM) and optimization

The extracts were obtained by dynamic maceration of powdered curcuma rhizomes. In the statistical design of experiments, a 2⁵ full factorial design was used. The experiments were carried out in an extractive system composed by a borosilicate vessel with 100 mL in volume and a glycerin bath, which was mounted on a stirring hot plate TE-085 (Marconi Ltda, Piracicaba, SP, Brazil).

The factors studied (independent variables) and their levels were: extraction time, Et (12 and 24 h); agitation speed, As (30 and 70 rpm); drug to solvent weight ratio in dry basis, DSr (1/6 and 1/4, g/g); extraction temperature, T (50 and 80

°C) and ethanolic strength, ES (70 and 96%, v/v). Process variables were selected based on preliminary experiments. The dependent variables were the contents of soluble solids and curcumin in the extracts obtained. Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The temperature of the extractive solutions were measured and adjusted using a thermometer (Incoterm Ltda, Porto Alegre, RS, Brazil) and the stirring speed with a optic tachometer TO 404 (Takotron Ltda, São Paulo, SP, Brazil). The factors were coded to allow the analysis of variance (ANOVA) by the RSM following the coding rule given by equation (1):

$$\text{Coded.value} = \frac{(\text{uncode.value} - 0.5 \times (\text{high.value} + \text{low.value}))}{0.5 \times (\text{high.value} - \text{low.value})} \quad (1)$$

ANOVA/RSM on the experimental data was performed using the software Design Expert® 7.0 (Stat-Ease® Inc., Minneapolis, MN, USA). The mathematical models for each response were evaluated using a multiple regression method. The response function applied was a linear polynomial equation, given by equation (2):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum \beta_{ij} x_i x_j \quad (2)$$

In equation (2), Y is the dependent variable; β_0 is the constant term; k number of variables; β_i represents the coefficients of linear parameters; β_{ij} represents the coefficients of interaction parameters.

The significance of the equation parameters for each response variable was analyzed by F-test. Only the factors with significance higher than or equal to 5% ($p \leq 0.05$) were considered. The model adequacy was checked accounting for the coefficient of determination (R^2). Finally, simultaneous optimization of the multiple responses was performed using the same software. All the independent variables were kept within range while the responses were either maximized, being attributed the same importance.

HPLC-PDA curcumin quantification

HPLC analysis was performed on a LC system comprising a quaternary pump (LC-10AT), a degasser (DGU-10A), a manual sampler (SIL 10A) and a SPD-10A photodiode array (PDA) detector (Shimadzu®, Kyoto, Japan). Chromatographic separation was carried out with a Lichrosorb® CN column (250 mm x 4.0 mm, 10 µm) purchased from Merck® (Merck KGaA, Darmstadt, Germany). The mobile phase,

which was composed of 50% acetonitrile and 50% citric acid aqueous solution (1.0% v/v), had the pH adjusted to 3.0 with a NaOH (0.2M) aqueous solution and was set at an isocratic mode with a flow rate of 1.0 mL/min. The detection wavelength was 425 nm. The injection volume was 50.0 µL and the total run time was fixed at 20 min. Data acquisition and analysis were performed by using a Shimadzu® Controller Module (CBM-20A Prominence) coupled to a computer with Shimadzu® LC Solution software. Reliability of the analytical method was assessed by a single laboratory validation study performed according to Agência Nacional de Vigilância Sanitária (Brazilian National Health Surveillance Agency) guidelines (Anvisa, 2003). The method presented linearity over the range from 2.5 to 300 µg/mL ($R^2=0.9998$); limit of detection and limit of quantification of 0.160 µg/mL and 0.169 µg/mL, respectively; suitable selectivity; accuracy of 94.7%; within-and between day precisions with relative standard deviation values of 1.6 and 2.98%, respectively.

The calibration curve was constructed by the dilution of curcumin standard (Sigma-Aldrich Co., Steinheim, Germany) with ethanol to provide the desired concentrations (2.5, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 100.0 µg/mL) followed by injection into the HPLC system. Samples were directly dissolved in 95% ethanol to reach the concentration of 40.0 µg/mL. Prior to injection in the LC system, both standard solutions and samples were filtered through 0.45 µm Millex® (Millipore, São Paulo, SP, Brazil) membranes. The extracts soluble solids contents (SSc, %) in dry basis were measured from 0.5 g of sample employing a halogen lamp moisture analyzer MB 45 (Ohaus Inc., USA). The curcumin content (Cc, %) in dry basis was calculated based on dried mass of extractives (% d.b.) for each extract. The soluble solids yield and curcumin yield were calculated according to equations 3 and 4, respectively:

$$\text{Soluble solids yield (\%)} = \frac{\text{soluble solids (g)} \times 100}{\text{turmeric used (g)}} \quad (3)$$

$$\text{Curcumin yield (\%)} = \frac{\text{curcumin extracted (g)} \times 100}{\text{turmeric used (g)}} \quad (4)$$

Results and Discussion

Pharmacognostic characterization of turmeric

The moisture content in the powdered material was 9.82 ± 0.15 (% w/w). This value is consistent with that specified in the Farmacopéia Brasileira (2010)

which consider as acceptable values lower than 12%. The residual moisture is an indicator of the efficiency of the processing and conservation and has considerable effect on the chemical and microbiological stability of the products. The total ashes content was 7.4 ± 0.045 (% w/w). Regarding this parameter, the Farmacopéia Brasileira (2010) proposes as acceptable values below 8%. Total ash contents above the established level indicate the presence of non-volatile inorganic impurities that may be present as contaminants or adulterants on the herbal material.

A swelling index of 4.72 ± 0.07 was observed. The powder particle size distribution assay revealed that the powder was moderately coarse, since around 37% of the particles passed through the sieve with mesh 1mm (Farmacopéia Brasileira, 2010). From a phytopharmaceutical technology point of view, processing an herbal raw material to reach a suitable degree of comminution is mandatory for the development of intermediate products under optimized conditions, since particle size determines the surface area available for the diffusional mass transfer of actives from drug to the solvent.

Effects of extraction parameters

The results of dynamic maceration experiments are summarized in Table 1. Under the established conditions, the SSc values ranged from 0.8 to 3.4%, while Cc ranged from 0.1 to 1.8%. These values correspond to soluble solids yields ranging from 4.1 to 14.1% and curcumin yields ranging from 2.1 to 62.6%. The higher curcumin yield was obtained with an extraction time of 24 h, agitation speed of 70 rpm, drug to solvent ratio of 1/6, extraction temperature of 80 °C and ethanolic strength of 96% (run. 28). The curcumin yield was higher in the present study as compared to previous one (Sogi et al., 2010), in which were obtained curcumin yields ranging from 4.5 to 12.9 %. It might be due to the different composition of curcuma (different sources), extraction condition and analytical technique employed in the curcumin quantification, since Sogi et al., (2010) used spectrophotometry.

Many factors such as the extractive method, solvent composition, extraction time, extraction temperature, solvent to drug ratio and extraction pressure, among others, are assumed to significantly influence the efficiency of curcumin extraction (Wakte et al., 2011). Accordingly, it is more adequate to use an optimization method that can take all the factors in account.

The tables with complete ANOVAs for each dependent variable are omitted, but a summary of the RSM analysis is listed in Table 2 where the levels of significance are displayed as percentages. As can be

Table 1. Results of extracts characterization.

Run order*	Standard order**	X_1 , Et	X_2 , As	X_3 , DSr	X_4 , T	X_5 , ES	SSc	Cc	SSY	CY
8	1	12 (-1)	30 (-1)	1/6 (-1)	50 (-1)	70 (-1)	1.9	1.0	8.9	35.3
20	2	24 (1)	30 (-1)	1/6 (-1)	50 (-1)	70 (-1)	1.3	1.0	5.3	33.8
24	3	12 (-1)	70 (1)	1/6 (-1)	50 (-1)	70 (-1)	2.4	1.1	11.1	37.4
2	4	24 (1)	70 (1)	1/6 (-1)	50 (-1)	70 (-1)	2.6	0.1	7.7	5.0
10	5	12 (-1)	30 (-1)	1/4 (1)	50 (-1)	70 (-1)	2.7	1.1	7.6	36.6
25	6	24 (1)	30 (-1)	1/4 (1)	50 (-1)	70 (-1)	3.2	0.8	8.2	29.2
11	7	12 (-1)	70 (1)	1/4 (1)	50 (-1)	70 (-1)	3.3	0.5	9.0	18.6
27	8	24 (1)	70 (1)	1/4 (1)	50 (-1)	70 (-1)	3.2	0.8	9.2	28.5
18	9	12 (-1)	30 (-1)	1/6 (-1)	80 (1)	70 (-1)	2.0	0.2	8.7	7.8
12	10	24 (1)	30 (-1)	1/6 (-1)	80 (1)	70 (-1)	2.5	0.1	10.1	2.1
29	11	12 (-1)	70 (1)	1/6 (-1)	80 (1)	70 (-1)	1.8	0.3	7.8	11.6
17	12	24 (1)	70 (1)	1/6 (-1)	80 (1)	70 (-1)	2.8	0.1	11.5	3.1
22	13	12 (-1)	30 (-1)	1/4 (1)	80 (1)	70 (-1)	2.7	0.8	7.7	26.8
28	14	24 (1)	30 (-1)	1/4 (1)	80 (1)	70 (-1)	2.9	0.2	6.1	7.5
34	15	12 (-1)	70 (1)	1/4 (1)	80 (1)	70 (-1)	3.4	1.0	14.1	34.2
30	16	24 (1)	70 (1)	1/4 (1)	80 (1)	70 (-1)	1.9	0.1	4.4	2.1
32	17	12 (-1)	30 (-1)	1/6 (-1)	50 (-1)	96 (1)	1.0	1.7	5.0	58.9
6	18	24 (1)	30 (-1)	1/6 (-1)	50 (-1)	96 (1)	0.8	1.7	4.1	57.7
4	19	12 (-1)	70 (1)	1/6 (-1)	50 (-1)	96 (1)	1.1	1.6	5.6	53.4
31	20	24 (1)	70 (1)	1/6 (-1)	50 (-1)	96 (1)	1.2	1.5	5.1	50.0
7	21	12 (-1)	30 (-1)	1/4 (1)	50 (-1)	96 (1)	2.3	1.4	7.1	47.9
3	22	24 (1)	30 (-1)	1/4 (1)	50 (-1)	96 (1)	2.1	1.5	6.4	50.1
16	23	12 (-1)	70 (1)	1/4 (1)	50 (-1)	96 (1)	1.8	1.6	5.5	54.1
1	24	24 (1)	70 (1)	1/4 (1)	50 (-1)	96 (1)	1.7	1.5	5.3	51.0
5	25	12 (-1)	30 (-1)	1/6 (-1)	80 (1)	96 (1)	1.0	1.7	5.0	57.1
13	26	24 (1)	30 (-1)	1/6 (-1)	80 (1)	96 (1)	1.2	1.6	5.8	53.6
26	27	12 (-1)	70 (1)	1/6 (-1)	80 (1)	96 (1)	1.6	1.7	7.7	56.5
14	28	24 (1)	70 (1)	1/6 (-1)	80 (1)	96 (1)	1.2	1.8	6.6	62.6
15	29	12 (-1)	30 (-1)	1/4 (1)	80 (1)	96 (1)	1.9	1.4	5.7	49.0
19	30	24 (1)	30 (-1)	1/4 (1)	80 (1)	96 (1)	2.0	1.4	6.1	48.7
9	31	12 (-1)	70 (1)	1/4 (1)	80 (1)	96 (1)	2.3	1.5	6.8	49.7
21	32	24 (1)	70 (1)	1/4 (1)	80 (1)	96 (1)	1.8	1.8	8.3	62.3

X_i : Coded factors in the experimental design; -1, 1: coded levels in the experimental design; Et: extraction time (h); AS: agitation speed (rpm); DSr: mass of drug to mass of solvent ratio (-); T: Extraction temperature (°C); ES: ethanolic strength (%); SSc: soluble solids contents (% d.b.); Cc: curcumin contents (% d.b.); SSY: soluble solids yield (% d.b.); CY: curcumin yield (% d.b.); *Randomized; **No randomized.

seen in Table 2, nor the soluble solid contents (SSc) neither the curcumin contents (Cc) were affected by the agitation speed. However, the extraction temperature (T) and ethanolic strength (ES) exerted a strong impact on the SSc, with 0.01% significance level. Using RSM, it was possible to access the contribution of these factors in the response, which accounted 38.9 and 45.2% for extraction temperature and ethanolic strength, respectively. The surface response plot of SSc as a function of the T and ES is presented in Figure 1. It can be observed in the response surface shown in

Figure 1 that increasing of T had a positive influence on SSc. On the other hand, increasing of ES had a negative influence on SSc, which means that the higher the ES, the lower the soluble solid content.

The effect of extraction factors on the curcumin contents, Cc, can be seen in Figure 2 and 3. As shown in Table 2, the curcumin contents proved to be dependent on the Et and ES at significant levels of 0.01 and 5%, respectively. When isolated, both Et and ES had a negative influence on Cc (Table 2). Furthermore, Cc depended on the interactions between Et and ES, and DSr

and ES, which exerted positive influence at significant levels of 0.1 and 5%, respectively. According to RSM analysis, Et and ES had contributions of 3.0 and 74.3% on the response, respectively, while the interactive terms Et x ES and DSr x ES had contributions of 5.0 and 3.0%, respectively. Is it clear from the results that the alcoholic proportion is the main factor involved in the extraction by dynamic maceration of curcumin from turmeric. It is also noteworthy to mention that high extraction times led to a decrease in the efficiency of the process. A plausible explanation for this behavior may be the occurrence of degradation of the curcumin in the whole extract.

Table 2. Summary of RSM analysis.

Coefficients	SSc	Cc
Intercept	-1.7131	4.3443
Et	0.0028	-0.2079 ^c
As	0.0528	-0.0318
DSr	10.0324	-17.1268
T	0.0703 ^a	0.0154
ES	-0.0098 ^a	-0.0135 ^a
Et x As	-0.0004	0.0006
Et x DSr	0.0174	-0.0397
Et x T	-0.0005	0.0005
Et x ES	0.0006	0.0017 ^b
As x DSr	-0.0714	-0.0119
As x T	-0.0002	0.0001
As x ES	-0.0002	0.0002
DSr x T	-0.0218	0.0179
DSr x ES	-0.0710	0.1843 ^c
T x ES	-0.0002	-0.0004
Model [#]	8.6 ^a	12.73 ^a
R ²	0.89	0.92

Significant at: ^a 0.01%; ^b 1%; ^c 5%; [#] F value; β_0 : model constant; R²: coefficient of determination; Et: extraction time (h); AS: agitation speed (rpm); DSr: mass of drug to mass of solvent ratio (-); T: Extraction temperature (°C); ES: ethanolic strength (%); SSc: soluble solids contents (% d.b.); Cc: curcumin contents (% d.b.).

The trends observed for the influence of ES on the curcumin contents match the trends observed for the soluble solid contents, which emphasizes the predominance of curcumin within the curcuminoids present in the sample. The expressive extraction of curcumin using ethanol 70% in the solvent mixture may be due to the higher dielectric constant of this solvent, when compared to the other proportion used (96%) (Jouyban et al., 2004).

RSM enables the fitting of polynomial equations of the dependent variables as a function of the

studied factors for predicting quality indicators (Table 2). The models adequacies were checked accounting for coefficient of determination. Coefficient of determination, R², is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for a good fitted model, R² should not be less than 80%. When R² approaches to the unity, means the suitability of fitting empirical model to the actual data. The lower value of R² shows the inappropriateness of the model to explain the relation between variables (Box et al., 1978).

As shown in Table 2, our results showed that the R² values for these response variables were higher than 0.80. This, together with the fact that the F values of fitted models were significant ($p > 0.05$), indicates that the regression models presented a great adjust, being suitable to predict the responses and explain their behavior. Sogi et al. (2010) also studied effect of extraction parameters on curcumin yield from turmeric using central composite rotatable design involving four variables (temperature, particle size, mixing time, and ethanol to meal ratio) at 5 levels and reported R² value of 0.78 between experimental and predicted values.

Extraction optimization

In further analysis of these responses, optimization was carried out using Design Expert 7.0 to obtain the criteria for maximum soluble solids and curcumin contents. By applying a desirability function method, a total of 30 solutions were found to satisfy the goal. The predicted optimized condition with higher desirability (69.1%) is shown in Table 3. This optimum condition provided soluble solid of 2.9% (d.b.) and curcumin contents of 1.1% (d.b.), which correspond to a soluble solid yield of 12.6% and a curcumin yield of 39.4%, respectively.

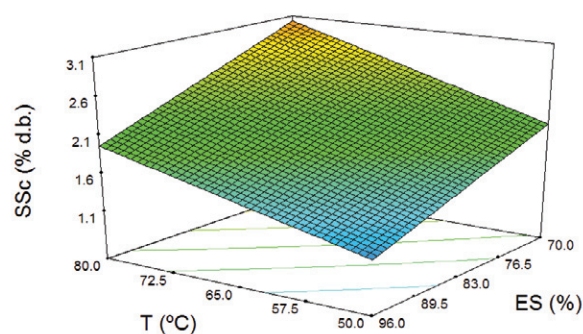


Figure 1. Surface response plot of soluble solid content as a function of extraction temperature and ethanolic strength in the solvent mixture.

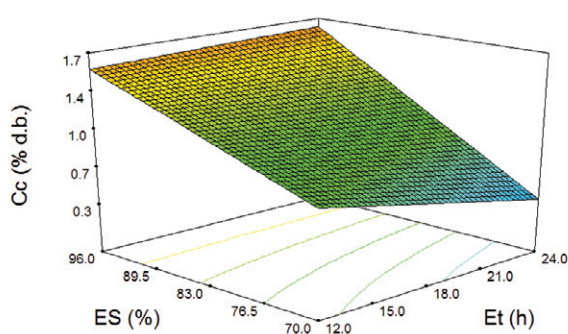


Figure 2. Surface response plot of curcumin content as a function of extraction time and ethanolic strength in the solvent mixture.

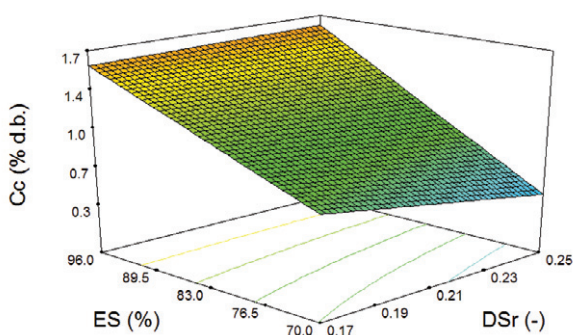


Figure 3. Surface response plot of curcumin content as a function of weight of drug to weight of solvent ratio and ethanolic strength in the solvent mixture.

Table 3. Predicted optimum condition for dynamic maceration extraction of curcumin from turmeric.

Factors	Low	High	Optimum
Et (h)	12	24	12
As (rpm)	30	70	30
DSr (-)	1/6	1/4	1/6
T (°C)	50	80	80
ES (%)	70	96	70

Et: extraction time; AS: agitation speed; DSr: mass of drug to mass of solvent ratio; T: Extraction temperature; ES: ethanolic strength of the solvent mixture.

Conclusions

The experimental design and RSM were successfully employed in the optimization of the dynamic maceration extraction of curcumin from *Curcuma longa* L., Zingiberaceae, rhizomes. ANOVA/RSM proved that studied factors, except the agitation speed, significantly affected the quality indicators at different levels. It was found that the alcoholic strength of the solvent mixture plays a crucial role in

the process efficiency. The responses were correlated with independent variables and the data points were fitted in linear models with significant F values and suitable values of R^2 . This work provides scientific evidences to the impact of in-process parameters on the dynamic maceration extraction of curcumin from *C. longa* rhizomes. Moreover, useful data for the further development of a phytopharmaceutical intermediate product with optimized characteristics were reported.

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