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Statistical modeling of a process for intensification of extraction of crude constituents of *Curcuma longa*

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Abstract

In order to intensify the extraction of crude constituents of *Curcuma longa*, three independent variables namely lactic acid (0.01-1)%, time (10-60) min and solid/solvent ratio (0.01-0.1) were concurrently studied using the central composite rotatable design on some crude extract characteristics namely; crude extract soluate, total phenolic content, pH and colour density as responses. Statistical modelling of the independent variables on the responses was attempted. The intensification process was characterised by high enhanced extraction of crude constituents (µg/mL) 67.00-440.00 and 28.85-133.17 in comparison to the low amount8.65-62.50 and 1.70-23.48 of crude constituent extract of the control sample measured at λ_{420} and λ_{520} respectively. Statistical modelling of the process was accomplished and equations for predicting the responses were developed and adequacy confirmed using analysis of variance and residual assessment. The intensified process could serve as economic driven route for the extraction of crude constituents of *C. longa* which could serve as a feed stock for preparation of value added curcuminoid based products with special dietary colourant and bioactive characteristics.

Keywords: *Curcuma longa*, extraction process variables, crude extract characteristics, statistical modeling

1. Introduction

Turmeric (*Curcuma longa)* L Zingiberaceae) is a good source of dietary colourants. The inherent colourant of turmeric is characterized with superior tinctorial strength in comparison to synthetic dye of similar shade for instance tartrazine yellow [1]. Besides, it has been used in treatment of spectrum of diseases such as heart disorder, liver problem, asthma arthritic, gall bladder infections, digestive disorders and dysmenorrhea [2]. Like any other natural product, preference for *C.longa* is based on natural originality, eco-friendly and renewability; hence there will be increase in utilization both in food and pharmaceutical industries. Beyond this, the active components of turmeric have been modified to enhance its antifungal and antibacterial [3] and anticancerous properties [4].

Similarly, the properties of *C longa* could be improved by the use of mordant and cationic complexation and other means such as application of cyclodextrin [5,6]. From the above stated, it suffice that crude constituents of *C.longa*, a precursor to all these is sparingly soluble in water at room temperature. Prior to this, study a facile process for the extraction of crude constituents of *C longa* had been modeled by Daramola [7]. Therefore, there is a need for a conceptual development of a process for intensification of extraction of the crude constituents of *C. longa* at room temperature, thus the subject of this study. Solubility of organic constituent can be enhanced by a number of methods notably, application of appropriate solvent in combination with size reduction, elevated temperature, and application of surface active agents.

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One or a combination of these could be used to intensify the extraction of crude constituents of natural products.

The objective of this study was to statistically model an intensification process for the extraction of crude constituents of *Curcuma longa* which could serve as feed stock for preparation of high value added products in nature of dietary colourant and bioactive products. The process should offer route for obtaining high yield crude extract of *C. longa* and associated economic implications.

2. Materials and Methods

2.1.Materials

Whole some *Curcuma longa* rhizome (red ginger) fingers were obtained from central commercial market in Ado-Ekiti, Nigeria. All chemicals used were of analytical grade: lactic acid, distilled water.

Curcuma longa crude constituents' extraction protocol. Curcuma longa rhizome without blemish was washed, peeled and dried. The dried product was milled and used in subsequent active-constituents dissolution process. Selected bioactive constituent recovery process was accomplished using two solvents mixture system, contact time, medium temperature as the dissolution independent variables. Details of extraction variables in the experimental design are presented in Table 1. The lowest and highest levels of independent variables were chosen from the results of preliminary investigations.

Experimental Design. A central composite rotatable design for k=3 was used [8]. The 3-factor, 5-level design generated 20 sample combinations comprising eight points peculiar to 2^3 factorial, six star points and six central points for replication. The effects of independent variables namely: Solvent-mixture ratio, temperature and time on dissolution of the selected crude active constituents. The crude active constituents markers namely total phenolic content, tinctorial (colour intensity) index and total soluble solids of solution were evaluated. Step-wise regression analyses were performed on the data to yield equations for predicting dissolution of active constituents of *Curcuma longa* with reference to designated functionality.

2.2.Analytical Methods

*Total soluble solids determination.*Refractive index of sample was measured using Abbey Refractometer (ABBE 325, ZUZI) and corresponding soluble solid was determined using appropriate procedure and reference to appropriate designated table.

Determination of colour density/polymeric colour. Colour density was determined according to the method described by Wrolstad et al [9]. Colour density was calculated as the sum of the absorbances at 420nm and 510nm.

*Evaluation of Total phenolic content***.**Total phenolic content was evaluated according to the method described by Taga et al [10]. Briefly, A 100µL of Folin – Ciocalteau reagent (2N wrt acid Fluka Chemic AG – Ch -9470 BUCHS) was added to each sample (20µL) and well mixed after addition of 1.58ml of water. After 30s, 300µL of 2 % sodium carbonate solution was added and the sample tubes were left at room temperature for 2h.The absorbance (A) of the developed blue colour was measured at 750 nm using Unicam Helios & uv / vis / spectrophotometer. A plot of A_{750nm} against corresponding concentration was used to calculate phenolic content (mg/g ascorbic acid equivalent).

2.3.Statistical analysis.

The central composite orthogonal designed was analysed as repleted by Cochran and Cox [8] (1957). Each of the X-matrix was multiplied by the Y-column (response) to obtain corresponding sums of products that is 0y to 13y for Xo toX_1 X3. Consequently, the coefficients b_0 to bi3 were calculate as:

$$b_0 = 0.166338(0y) - 0.056791\ \Sigma(iiy) \quad (1)$$

$$bi = 0.073224(iy) \quad (2)$$

$$bii = 0.062500(iiy) + 0.006889\Sigma\ (iiy) - 0.056791(0y) \quad (3)$$

$$bij = 0.125000(ijy) \quad (4)$$

The quadratic model was fitted using the regression coefficients and the predicted response calculated for each of the observed values. The model was observed for adequacy by subjection to analysis of variance and residual analysis.

3. Results and Discussion

Of all the acids screened, lactic acid showed the best result for intensification of extraction of crude constituents of *C. longa* at room temperature. Therefore the acid was employed for extraction of crude constituents of *C. longa* in this study. Influence of process variables namely, solid-solvent ratio, lactic acid concentration and contact time have been observed to exert profound influence on the extraction of crude constituents of *C. longa*.

The result of this investigation measured at λ_{420} and λ_{520} is shown in Table 2. Lactic acid, a food grade reagent was efficient in enhancing extraction of crude constituents of *C. longa* at different process conditions (concentration of acid, solid to solvent ration, time) as shown in Table 2.

The soluate and other crude extract constituent characteristics were subsequently statistically modelled.

Four technological parameters namely, extracted soluate, total phenolics content, colour density and pH were assessed the lactic acid treated samples and the sample with no lactic acid treatment served as the control (reference) for analytical discussion. The central composite orthogonal design to fit the polynomial model for the lactic acid in *C.longa* soluate dissolution intensification process was accomplished as elicited by Cochran and Cox [8]. The computed sums of products and regression coefficients to fit the model are shown in Table 3.

Dissolve soluate. The dissolve soluate quadratic model (DS) for the lactic acid enhanced *C.longa* crude component dissoluate takes the form:

$$DS = 276.45 + 3.075X_1 + 88.528X_2 + 20.06X_3 - 52.70X_1^2 - 3.39X_2^2 - 35.08X_3^2 - 7.625X_1X_2 + 16.375X_1X_3 + 25.125X_2X_3$$

The predicted dissolved soluates (DS) for each of the experimental runs and their respective residual are shown in Table 4. Examination of the residuals suggests that the fitted model was reasonably adequate. The claim was confirmed by model testing. In addition, the analysis of variance to test the fitness of the model is shown in Table 5.

The first and second order terms were significant as shown by the higher calculated F-ratio compared with the tabulated values. However, since the calculated F-ratio for the lack of fit was lower than the tabulated value therefore adequacy of the fitted model is affirmed.

Total phenolic content. The antibiotic activities of active constituent of *C. longa* are largely due to the phenolic content [1]. Therefore its extraction could also be influenced by the surfactant (lactic acid).

The total phenolic acid content quadratic model takes the form:

$$TPC = 6.256184 + 0.40295167X_1 + 0.387633X_2 + 1.510245X_3 - 0.930906X_1^2 - 0.3295936X_2^2 - 0.6284686X_3^2 + 0.0275X_1X_2 - 0.0725X_1X_3 - 0.0225X_2X_3$$

The predicted total phenolic content (TPC) for all the experimental runs and corresponding residual are presented in Table 4. Examination of the residuals infers that the fitted model was adequate: The conjecture was attested by model testing. Added to this, the analysis of variance to test the fitness of the model is shown in Table 5.

The first and second order terms were significant as shown by the higher calculated F-ratio compared with the tabulated values. However, since the calculated F-ratio for the lack of fit was lower than the tabulated value consequently, adequacy of the fitted model is affirmed C. *longa* dietary colourant has been pigment of choices because of its high colour density [1].

Colour Density. Colour density reflects the chromatically of the lactic acid enhanced dissolved bioactive components. The colour density (CD) quadratic model takes the form.

$$CD = 1.504126344 + 0.013085129X_1 + 0.22183943X_2 + 0.33945182X_2 - 0.29662662X_1^2 - 0.27188287X_2^2 - 0.0438766165X_3^2 + 0.02X_1X_2 + 0.0775X_1X_3 + 0.08X_2X_3$$

Both the residual analysis (Table 4) and analysis of variance (Table 5) showed the adequacy of the model. The model was also confirmed by model testing.

Table 1. Process variables used in the central composite rotatable design (K = 3)

Independent Variables	Code	Levels			
	-1.68	-1	0	1	1.68
Solvent/solid ratio ml:g (X_1)	1:0.01	1:0.02	1:03	1:0.06	1:0.1
Lactic acid (%)(X_2)	0.01	0.05	0.2	0.5	1
Time (X_3)	10	22	34	46	60

* = 100mL of (water) was used for each treatment

Table 2. Influence of process variables on extraction of crude constituents of *Curcuma longa* at λ_{420} and λ_{520}

Run	Treatment			λ_{420}			λ_{520}		
	X_1	X_2	X_3	Treated	Control	Difference	Treated	Control	Difference
1	-1	-1	-1	120.00	18.75	101.25	65.87	1.90	63.97
2	1	-1	-1	103.00	15.30	87.70	28.85	1.70	27.15
3	-1	1	-1	234.00	42.31	191.69	96.15	12.00	84.15
4	1	1	-1	187.00	35.58	151.42	65.87	8.65	57.22
5	-1	-1	1	67.00	09.14	57.86	38.94	1.92	37.02
6	1	-1	1	116.00	12.02	103.98	35.58	1.90	33.68
7	-1	1	1	282.00	52.59	229.41	133.17	15.38	117.79
8	1	1	1	300.00	40.65	259.35	72.60	8.65	63.95
9	-1.68	0	0	107.00	08.50	98.50	45.67	4.92	40.75
10	1.68	0	0	130.00	15.39	114.61	35.58	1.92	33.66
11	0	-1.68	0	76.00	8.65	67.35	28.85	1.92	26.93
12	0	1.68	0	440.00	50.00	390.00	106.25	15.38	90.87
13	0	0	-1.68	123.00	18.80	104.20	38.44	1.92	36.52
14	0	0	1.68	214.00	40.00	174.00	59.14	8.65	50.49
15	0	0	0	270.00	50.00	220.00	50.50	23.48	25.02
16	0	0	0	280.00	62.50	217.50	82.69	18.75	63.94

*µg solute/ml of solution, X_1 = time, X_2 = lactic acid, X_3 = solid-solvent ratio

Table 3. Regression Coefficient for the quadratic model equation for lactic acid intensity *Curcuma longa* solution

Sum of products	**Dissolve soluate**	**Phenolic content**	**Colour Density**	**pH**	**Regression coefficient**	**Dissolve soluate**	**Phenolic content**	**Colour density**	**pH**
0y	4104	99.33	21.72	55.50	Bo	267.45	6.2562	1.504126	2.4981
Y	42	5.503	0.1787	-0.12	b1	3.075	0.40295	0.013085	-0.00878
2y	1209	5.2938	3.0296	-0.79	b2	88.528	0.38763	0.221839	-0.057847
3y	274	20.625	4.6358	-6.66	b3	20.06	1.510245	0.339452	-0.487672
11y	2080	55.44	10.8972	38.91	b11	-52.70	-0.9309	-0.296626	0.0968
22y	2869	65.06	11.2931	40.61	b22	-3.39	-0.32959	-0.27188	0.203053
33y	2362	60.28	14.9412	39.05	b33	-35.08	-0.6285	-0.043877	0.105553
12y	-61	0.22	0.16	-0.05	b12	-7.625	0.0275	0.02	-0.00625
13y	131	-0.58	0.62	0.15	b13	16.375	-0.0725	0.0775	0.01875
23y	201	-0.18	0.64	0.35	b23	25.125	-0.0225	0.08	0.04375
Σ(ii)y	7311	180.78	37.1315	118.57					

Table 4. Residual analysis of assessed parameters

Expt.	Dissolve Soluate			Total Phenolic Content			Colour Density			pH		
Run	Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual
1	120.00	98.115	21.885	1.92	1.9900	-0.0700	0.50	0.4899	0.010	3.55	3.5141	0.0359
2	103.00	87.520	15.480	3.10	2.8928	0.2072	0.40	0.3261	0.074	3.50	3.4715	0.0285
3	234.00	240.175	-6.175	3.20	2.7640	0.4360	0.99	0.8185	0.172	3.20	3.3323	-0.1200
4	187.00	199.075	-12.075	3.80	3.7700	0.0300	0.76	0.6497	0.110	3.20	3.2557	-0.0560
5	67.00	55.985	11.020	5.00	5.2090	-0.2090	0.65	0.8588	-0.209	2.50	2.4136	0.0864
6	116.00	109.380	6.620	5.20	6.0100	-0.8100	0.65	0.9999	-0.349	2.60	2.4460	0.1540
7	282.00	298.545	-16.545	5.50	5.8850	-0.3850	1.28	0.7249	0.525	2.40	2.3980	0.0020
8	300.00	321.445	-21.450	6.50	6.6005	-0.1005	1.54	1.6430	-0.103	2.40	2.8444	-0.4400
9	107.00	113.242	-6.242	3.00	2.9448	0.0552	0.67	0.6433	0.027	2.80	2.7866	0.0130
10	130.00	123.586	6.414	4.50	4.3000	0.2000	0.80	0.6873	0.113	2.70	2.7571	-0.0571
11	76.00	108.956	-32.956	5.00	2,9440	2.0560	0.60	0.3627	0.238	3.00	3.1690	-0.1690
12	440.00	406.771	33.230	5.90	5.9760	-0.0760	1.01	1.1080	-0.098	3.10	2.9748	0.1250
13	123.00	134.503	-11.500	1.50	1.9300	-0.4000	0.50	0.8091	-0.309	3.70	3.6169	0.0830
14	214.00	201.985	12.020	7.71	7.0100	0.7000	2.40	1.9510	0.449	1.85	1.9760	-0.126
15	270.00	267.450	2.550	5.50	6.2500	-0.7500	1.50	1.5041	-0.004	2.60	2.4981	0.1020
16	280.00	267.450	12.550	7.00	6.2500	0.7500	1.00	1.5041	-0.504	2.70	2.4981	0.2019
17	270.00	267.450	2.550	6.00	6.2500	-0.2500	1.80	1.5041	0.296	2.60	2.4981	0.1019
18	250.00	267.450	-17.450	7.00	6.2500	0.7500	1.80	1.5041	0.296	2.30	2.4981	-0.1980
19	285.00	267.450	17.550	6.00	6.2500	-0.2500	1.80	1.5041	0.296	2.30	2.4981	-0.1980
20	250.00	267.450	-17.450	6.00	6.2500	-0.2500	1.10	1.5041	-0.404	2.50	2.4981	0.0019

Residual = Observed – Predicted

Table 5. Analysis of Variance (ANOVA) for the Predictive Model Equations

Dependent	Statistical term variable	DF	SS	MS	F_{ratio} Calc	F_{ratio} Tab 5%	F_{ratio} Tab 1%
Dissolved soluate	First order	3	112656.53	37552.18	65.025	5.41	12.06
	Second order	6	60841.68	10140.28	17.659	4.95	10.67
	Lack of fit	5	4551.49	910.30	1.576	5.05	10.97
	Error	5	2887.5	577.5			
	Total	19	180937.2				
Total Phenolic Content	First order	3	35.4183	11.806	31.48	5.41	12.06
	Second order	6	17.17	2.8617	7.63	4.95	10.67
	Lack of fit	5	1.727	0.3454	0.92	5.05	10.97
	Error	5	1.875	0.375			
	Total	19	56.1940				
Colour density	First order	3	2.248099	0.749366	18.73	5.41	12.06
	Second order	6	2.22578	0.370963	9.27	4.95	10.67
	Lack of fit	5	0.664	0.1328	3.32	5.05	10.97
	Error	5	0.200	0.04			
	Total	19	5.33788				
.Ph	First order	3	3.294647	1.098216	49.918	5.41	12.06
	Second order	6	0.782178	0.13036	5.9255	4.95	10.67
	Lack of fit	5	0.155673	0.03113	1.415	5.05	10.97
	Error	5	0.11	0.022			
	Total	19	4.3425				

DF = Degree of freedom, SS = Sum of square, MS = Mean square

pH. pH is a rapid objective physiochemical property. It could be an important quality control tool in place of elaborate analysis like total phenolic content and dissolved soluate in an established C.*longa* crude constituent extraction process.

The pH quadratic model takes the form:

$$\text{pH: } 2.4981 - 0.0087688X_1 - 0.05784696X_2 - 0.4876718X_3 + 0.0968X_1^2 + 0.203053X_2^2 + 0.01875X_1X_3 + 0.04375X_2X_3$$

The pH of the control C. *longa* extract was 6.58. This is similar to an earlier report elicited by Henry [1]. The predicted pH of the dissolved runs and their respective residual are shown in Table 4. Examination of the residuals suggests that that fitted model was reasonably adequate. The claim was confirmed by model testing. Added to this the analysis of variance to test the fitness of the model is shown in Table 5. The first and second order terms were significant as revealed by the higher calculated F-ratio compared with the tabulated values. However, since the calculated F-ratio for lack of fit was lower than the tabulated value therefore adequacy of the fitted model is affirmed.

4.Conclusion

Application of miniscule amount of lactic acid and other two physical variables namely particle size and contact time to intensify the extraction of crude constituents of *C. longa* was accomplished in this study. The model developed was found adequate for provide a process for enhancing extraction of crude consistent of *C. longa,* a feed stock for preparation of specialized curcuminoids bioactive components.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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