Nigerian Research Journal of Chemical Sciences, Vol. 6, 2019

Optimization of Ethanol-Water Extraction of Natural Colour from the Seeds of Bixa Orellana L. Plant Using Response Surface Methodology

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ABSTRACT

This study reported the ethanol-water extraction of natural colour from *Bixa orellana* L. plant seeds. Response surface methodology was used to investigate the effect of variables on the yield of extraction. The independent variables studied were time of extraction (30-150 min), temperature (30-70℃), weight of seed (1-7g) and ethanol volume (10-50 ml). According to the results, optimal colour yield was obtained at extraction time of 96 min, temperature of 60℃, seed weight of 2.5g and ethanol volume of 40 ml. At these optimized conditions, extraction yield of 11.7% was obtained.

Keywords: *Bixa orellana* L., ethanol, optimization, response surface methodology, water.

INTRODUCTION

Natural colours obtained from plants, animals and minerals were being used since prehistoric times to colour food substances [1], in dyeing, printing, painting and in the production of cosmetics [2], and also as an integral part of the cultural heritage of Africa used for art, symbolic/religious role, medicinal purpose etc.

Bixa orellana is a small tree that is grown in the tropics for ornamental purposes. The seed contains a mixture of eight colourants of carotenoid group. The major colourants are Bixin and Norbixin [3], having colour shades of yellow, yellow-orange and orange. Their major use is in the colouring of food, pharmaceutical and cosmetic products. Bixin has been reported to be the second most important natural colourant in the world [4]. Colour is obtained by leaching the pericarp of the seeds with an extractant prepared from one or more food grade materials like various organic solvents, edible vegetable oils and fats, and alkaline aqueous or alcoholic solution. The selection of an extractant is done depending upon the intended use [4].

The yield of natural colour extracted from *Bixa orellana L.* plant is significantly affected by the operating parameters, such as extraction time, extraction temperature, weight of seed, solvent

volume, etc [5]. However, the effects of the operating parameters and the interactions between them on the yield of ethanol-water colour extraction from the seeds of *Bixa orellana* L. plant have not been fully investigated. This research work aims to find the optimum conditions and also establish the effect of these operating parameters on the yield of natural colour extraction from *Bixa orellana* seeds.

MATERIALS AND METHODS

Experimental design

Four variables, five level central composite designs were chosen to design the experiment. The design consisted of 16 factorial points, 8 axial points and 6 center points. The four independent variables and their levels are shown in Table 1.0. A mathematical model describing the relationship between the response and the variables in second order equation was developed. The yield of extraction was regressed with respect to the conditions by the least square method as follows [6]:

$$y_{i=}\beta_i + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta\, i_j x_i x_j \ldots\ldots\ldots\ldots\ldots\ldots 1.0$$

Where $\beta_\circ$, β_i, β_{ii}, β_{ij} are constant regression coefficients of the model, and x_i, x_j (i=1,4;j=1,4,i≠j) represent the independent variables in the form of coded values. The accuracy and general ability of the polynomial model could be evaluated by the coefficient of determinationR^2. Design expert 6.0 was used for the design and analysis of the obtained result.

Table 1.0: Experimental range and levels of the independent variables of natural colour extraction from *Bixa orellana* seeds

	RANGE AND LEVEL				
INDEPENDENT VARIABLE	-2	-1	0	1	2
Extraction time, X_1, (min)	30	60	90	120	150
Extraction temperature, X_2, (℃)	30	40	50	60	70
Weight of seed, X_3, (g)	1	2.5	4	5.5	7
Ethanol Volume, X_4, (ml)	10	20	30	40	50

Colour Extraction

Ripe, dried fruits of *Bixa orellana* were harvested. The thorny pods containing the seeds were broken with hand. The seeds were separated from other particles.

Colour was extracted from the seeds by using a mixture of 50 ml water-ethanol. The process parameters that were optimized are weight of seed (1-7g), time of extraction (30-150 min), temperature (30-70℃), and ethanol volume (10-50 ml).

Bixa orellana seeds (1-7 g) were placed into a 250 ml conical flask and 50ml ethanol-water mixture was added, and then heated for selected temperature (30-70℃) for different periods of time (30-150 min). After extraction, the mixture was separated by filtration with a filter paper and dried on a heating mantle at 50℃.The yield of extracts after drying was measured using electronic balance and is represented as [7]:

$$Yield\ (\%) = \frac{weight\ of\ dried\ extract}{weight\ of\ seed\ taken} \times 100 \qquad (2.0)$$

RESULTS AND DISCUSSION

Model equations for natural colour yield

A central composite design was used to investigate the effect of four independent variables: time, temperature, seed weight and ethanol volume on the yield of natural colour extracted from *Bixa orellana* plant seeds. A total of 30 experiments were performed, with all experiments performed in duplicate. The result of experimental runs is presented in Table 2.0. At different combinations of the variables, natural colour yield varied between 1.2 (%, w/w) and 11.8 (%, w/w).

Table 2.0: Central composite design matrix and the response values

Run	A	B	C	D	Yield (%, w/w)
1	-1	-1	-1	-1	2.332
2	-1	-1	+1	-1	1.229
3	-1	+1	-1	-1	6.78
4	-1	+1	+1	-1	6.268
5	+1	-1	-1	-1	3.341
6	+1	-1	+1	-1	3.284

7	+1	+1	-1	-1	9.129
8	+1	+1	+1	-1	7.664
9	-1	-1	-1	+1	3.082
10	-1	-1	+1	+1	2.973
11	-1	+1	-1	+1	11.84
12	-1	+1	+1	+1	10.056
13	+1	-1	-1	+1	5.872
14	+1	-1	+1	+1	4.971
15	+1	+1	-1	+1	10.595
16	+1	+1	+1	+1	9.735
17	-2	0	0	0	4.826
18	+2	0	0	0	9.863
19	0	-2	0	0	2.83
20	0	+2	0	0	7.391
21	0	0	-2	0	10.372
22	0	0	+2	0	6.995
23	0	0	0	-2	3.775
24	0	0	0	+2	8.514
25	0	0	0	0	8.95
26	0	0	0	0	9.283
27	0	0	0	0	9.73
28	0	0	0	0	9.352
29	0	0	0	0	10.113
30	0	0	0	0	9.871

Based on experimental data, the developed quadratic model, in terms of actual variables, is given in Equations 3.0,where A, B, C and D represent extraction time, temperature, seed weight and ethanol volume, respectively.

$$Yield = -57.62 + 0.24A + 1.48B + 1.31C + 0.59D - 0.012B^2 - 0.14C^2 - 0.01BC \quad (3.0)$$

Analysis of variance (ANOVA)

The responses obtained (Table 2.0) were subjected to an analysis of variance, R-squared and lack-of-fit (LOF). Table 3.0 presents the analysis of variance (ANOVA).

From Table 3.0, model F-values of 10.78 implies the model is significant. Values of "Prob>F" less than 0.05 indicates model terms are significant. In this case A, B, C, D, A^2, B^2, and D^2 are significant model terms. The analysis of variance showed that there was a non-significant lack-of-fit (LOF F-values of 12.79), which further validates the model. The coefficient of determination R-squared was statistically high at 0.9096 which also reflected the degree of fit of the model.

Table 3.0: Analysis of variance (ANOVA) for the responses

Source	Sum of Squares	DF	Mean Square	F Value	Prob>F
Model	246.89	14	17.63	10.78	<0.0001
A	16.24	1	16.24	9.93	0.0066
B	121.97	1	121.97	24.59	<0.0001
C	7.64	1	7.64	4.67	0.0472
D	34.02	1	34.02	20.81	0.0004
A^2	12.46	1	12.46	7.62	0.0146
B^2	40.16	1	40.16	24.56	0.0002
C^2	2.75	1	2.75	1.68	0.2141
D^2	28.84	1	28.84	15.19	0.0014
AB	2.01	1	2.01	1.23	0.2849
AC	3.164E-003	1	3.164E-003	1.935E-003	0.9655
AD	0.80	1	0.80	0.49	0.4939
BC	0.38	1	0.38	0.23	0.6387
BD	2.01	1	2.01	1.23	0.2849
CD	0.017	1	0.017	0.010	0.9208
Residual	24.53	15	1.64		
Lack of fit	23.61	10	2.36	12.79	0.0058
Pure error	0.92	5	0.18		
Cor total	271.41	29			

Response surface analysis for natural colour yield

Figures 1.0-3.0 are response surface plots. The response surface plot is a 3D graphical representation of the regression equation. Each figure presented the effect of two variables on the yield of extracted natural colour while the other two variables were held constant at the middle level. For the purpose of this study, extraction time was increased from 30 min to 150 min at an

interval of 30 min, temperature from 30℃ to 70℃, seed weight from 1 g to 7 g, and ethanol volume from 10 ml to 50 ml.

The interaction term of time and temperature showed a significant effect on natural colour yield, which is illustrated in Figure 1.0 at zero level of seed weight (4.0 g) and ethanol volume (30 ml).

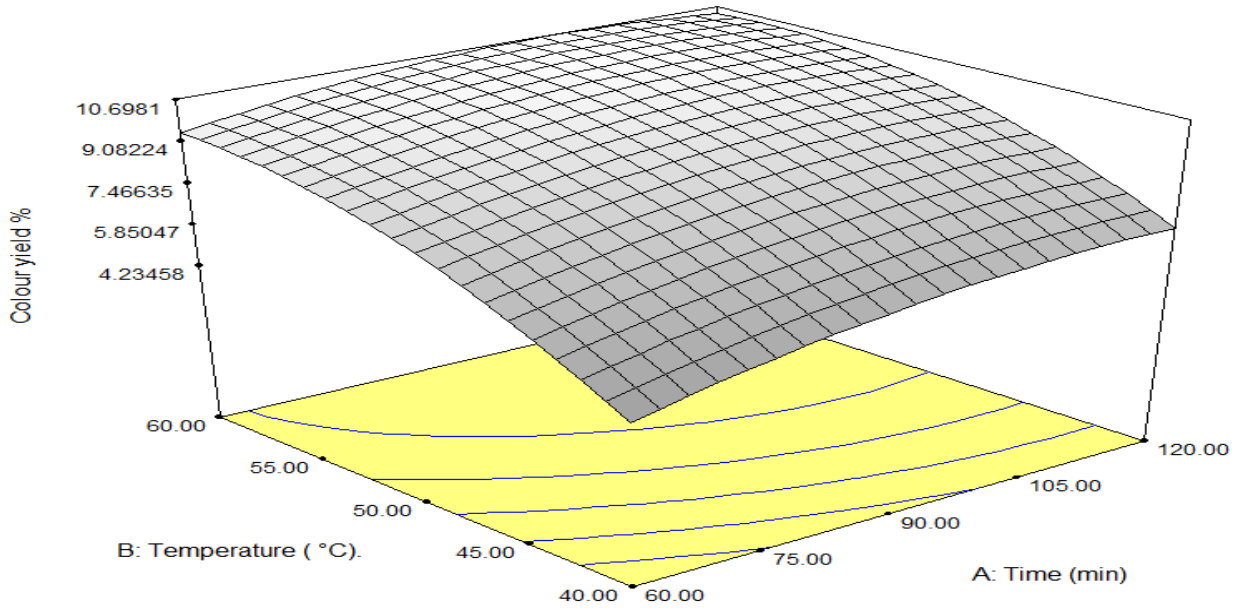

Figure 1.0: Effect of time and temperature on the yield of colour at constant seed weight (4 g) and ethanol volume (30 ml)

Analysis of this figure showed that at any designate time from 60-120 min, increasing the temperature from 40-60℃ resulted to an increase in yield from 4.2 (%, w/w) to 9.2 (%, w/w). However, at any designate temperature, yield is slightly affected by any change in time.

Figure 2.0 presents the effect of time and ethanol volume on the yield of natural colour at constant temperature and seed weight. This result showed that higher colour yield is enhanced by an increase in both extraction time and ethanol volume. As presented in the figure, natural colour yield increased with increasing ethanol volume. At any designated time from 60-120 min, natural colour yield increased from 5.7-8.2 (%, w/w), with increasing ethanol volume from 20-40 ml. Highest yield of 9.8 (%, w/w) was obtained at the maximum ethanol volume of 40 ml and time of 120 min.

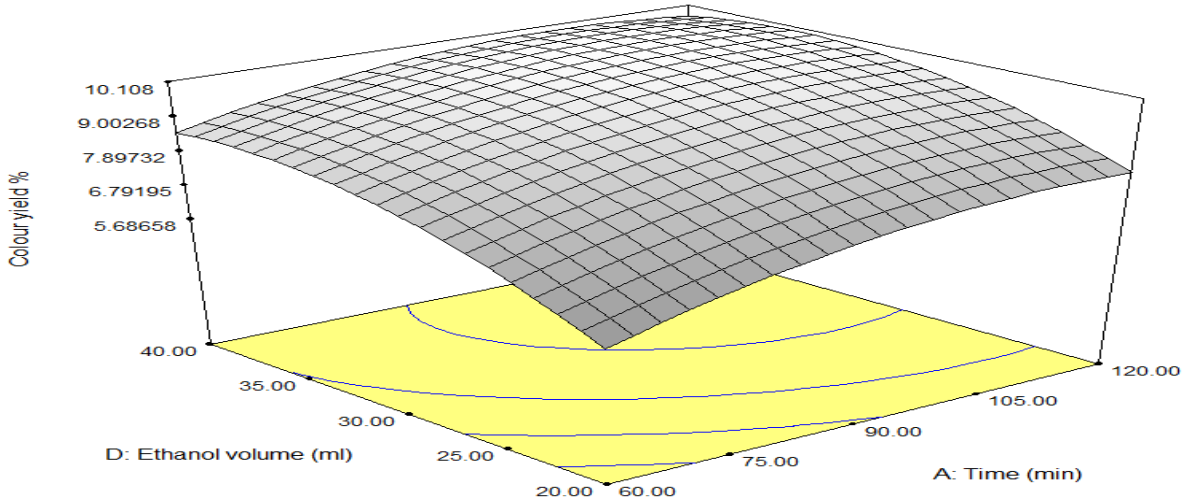

Figure 2.0: Effect of time and ethanol volume on the yield of colour at constant temperature (50℃) and seed weight (4 g)

It was observed that increase in the concentration of ethanol in the solvent mixture enhanced natural colour yield. This could be attributed to the fact that *Bixa orellana* seed colour dissolves poorly in water and more in ethanol [8], which is highly volatile and has a higher penetration capacity that could be increased with increase in temperature [9].

The effect of extraction time can be correlated with the higher contact time of solvent with the seed which grasped more colouring component into the solvent [10]. The longer the contact time between solvent and material, the more colour is extracted. The extraction period can, however, be shortened by either reducing the particle size, shaking the plant material-solvent mixture, or the use of non-conventional method of extraction.

The interaction effect of seed weight and ethanol volume on natural colour yield is presented in Figure 3.0. Colour yield is enhanced by an increase in ethanol volume from 20-40 ml with an increase in seed weight from 2.5-4.0 g.

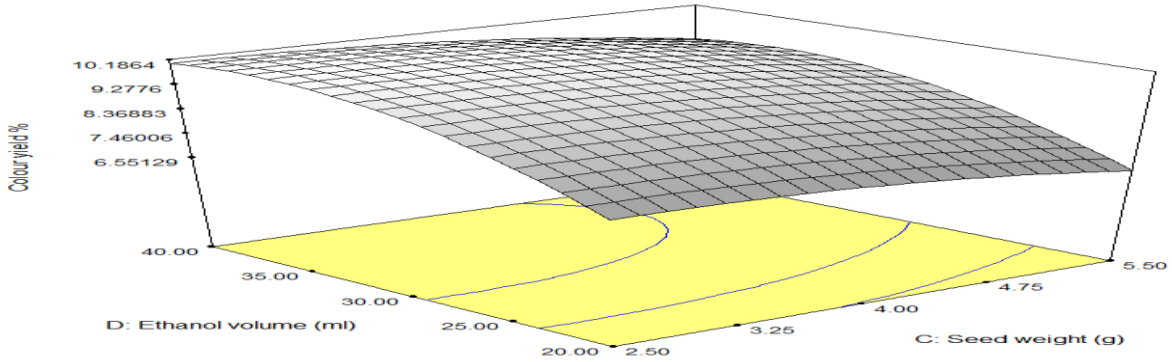

Figure 3.0: Effect of seed weight and ethanol volume on the yield of colour at constant time (90 min) and temperature (50℃)

In summary, maximum natural colour yield was obtained at a temperature of 60℃, time of 90 min, solvent volume of 40 ml, and seed weight of 4.0 g.

Optimization

The optimal values of the selected variables were obtained by solving the regression equation (Equation 3.0) using the optimization module of the Design-Expert 6 (RSM) software. The optimal conditions for the extraction of colour estimated by the model equation were as follows: 96.07 min time, 60℃ temperature, 2.5 g seed weight, and 38.04 ml ethanol volume.

The theoretical yield predicted under the above conditions is y = 11.7 (%, w/w).

CONCLUSION

A central composite design was used to investigate the effect of four independent variables: time, temperature, seed weight and ethanol volume on the yield of natural colour extracted from *Bixa orellana* plant seeds. The following conclusions were drawn from the study:

1. Colour yield was found to be a function of time, temperature, seed weight and ethanol volume and it increases with increase in the variables.
2. Optimization model was developed using RSM technique for prediction of colour yield from *Bixa orellana* plant seed. The model was found to fit experimental data.
3. Conditions of optimum colour yield were 96 min, 60℃, 1:16 seed/solvent ratio and 1:4 water/ethanol ratios. The yield under these conditions was 11.7 (%, w/w). .

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