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Valorization of Red Sorghum bran (*Sorghum bicolour*) for Anthocyanin Production: Yield, Optimization and Kinetics Study

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Abstract
Sorghum, an ancient cereal grain and a staple food for most families in Africa is rich in natural anthocyanin. Despite its high content of natural anthocyanin, the bran is discarded as waste and the possibility of anthocyanin extraction from it has not been given much attention. This work therefore aimed at evaluating anthocyanin extraction from red sorghum bran, and the influence of operating factors on optimizing its yield. Anthocyanin was extracted from sorghum bran using ethanol, and the extract dried and characterized for its functional groups by Fourier Transformed Infrared (FTIR) spectroscopy. Box Behnken Design of Response Surface Methodology (RSM) was employed to evaluate the effect of extraction parameters on anthocyanin concentration and yield. Bulk extraction gave anthocyanin concentration (20.05 g/L) and yield (0.09 g anthocyanin/g sorghum bran). FTIR spectra of the extract showed isothiocyanate functional groups confirming anthocyanin in the extract. Optimization gave maximum anthocyanin concentration (38.39 g/L) and yield (0.66 g anthocyanin/g sorghum bran) at the optimal condition of solvent volume;65.1 ml, temperature;75 ^{o}C and extraction time; 30.18 min. Results showed red sorghum bran as a potential source of anthocyanin and its extraction an eco-friendly means of wastes valorization.

Keywords: Red sorghum bran, Extraction, Anthocyanin, Characterization, Valorization.

Introduction
Sorghum, (*Sorghum bicolor*) is an ancient cereal grain plant of the grass family with edible starchy seeds. It's the fifth most produced cereal crop in the world, with an annual production of around 57.6 million tons (IIFPT, 2021). It is high in carbohydrates, protein, fat, calcium with small amounts of iron, vitamin B_1 and niacin. In addition, sorghum is high in antioxidants like flavonoids, phenolic acids, and tannins (Xu et al., 2021). The grain is usually ground into meals such as porridge, flatbreads, and cakes. It is also used for making edible oil, starch, dextrose, paste, and alcoholic beverages (Lazaro and Favier, 2000). Whole sorghum grain consists of 3 main parts: bran (the hard outer layer of the grain that contains fiber, minerals and antioxidants), germ (the nutrient-rich core that contains carbs, fats, proteins, vitamins, minerals, antioxidants and various phytonutrients) and endosperm (the biggest part of the grain that contains mostly carbs in the form of starch and protein). For human consumption, sorghum grain is dehulled either through wet-milling process or dehulling machine. Sorghum bran (SB), a by-product of sorghum grain dehulling is normally discarded as waste. Part of the bran is fed to animals while larger percentage ended up in the environment as solid waste which upon decomposition generate organic waste.

Colorants used in the food, pharmaceutical, cosmetic and textile industry are of two types, natural and synthetic (Baishya *et al.*, 2012). Natural colorants are obtained from plant materials while synthetic colorants are produced from chemicals. The major component of natural colorants is anthocyanin. Anthocyanins and anthocyandins are group of natural pigments produced by plants and responsible for colors of many leaves, flowers, seeds, grains and fruits (Pervaiz *et al.*, 2017; Bendokas *et al.*, 2020). They are colored molecules having medium-size and belonging to the class of flavonoids (Khoo et al., 2017). They are produced in a branch of phenylpropanoid pathway that involved biosynthesis of other flavonoids (Holton and Cornish, 1995). Apart from physiological roles in plants, anthocyanidins and

anthocyanins have been reported to play important roles in human health and well-being (Dini *et al*., 2018).

The intake of anthocyanin through consumption of foods rich in flavonoid compounds have been linked to an improvement of redox balance in humans due to their high scavenging and reducing activities (Mannino *et al.,* 2019; Jakobek *et al.,* 2007). Anthocyanins and anthocyanidins compounds are able to exert a wide range of biological and pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, and anti-atherosclerotic activity (Swallah *et al.,* 2020). Properties such as antitumor, antiatherogenic, antiviral, and anti-inflammatory effects, decrease of capillary permeability and fragility, inhibition of platelet aggregation and immune stimulation of anthocyanidins and anthocyanins were reported (Smeriglio *et al*., 2016).

Studies have shown the effectiveness of solid-liquid extraction process for separation of a wide variety of compounds from different sources (Kayode *et al.* 2011). The choice of solvent for extraction process depends on the capacity of solvent to dissolve the solute, temperature of extraction and the particulate size (Zhang et al., 2018). Hence this study aimed to investigate effect of operating conditions on the concentration and yield of extracted anthocyanin from red *Sorghum bicolor* bran. Box-Behnken design (BBD) of the Response Surface Methodology (RSM) was employed to evaluate process conditions that maximize anthocyanin concentration and yield from red sorghum bran. The effects of extraction conditions for optimum process conditions and kinetics of the process were then determined.

Materials and Methods

Material collection and preparation

Red sorghum grain purchased from a market in Ogbomoso, Nigeria, was cleaned thoroughly by blowing off chaff, dust and foreign materials. The grain was then processed to obtain bran according to procedure of Ange *et al.* (2015). Red sorghum grain was poured into a dehuller machine from which bran was separated from the grain. The bran was then dried at 50 ^{o}C for 4 hr in the oven, milled and screened to the range 0.5 - 2 mm particle size, then vacuum-packed in polyethylene bag prior to experimentation.

Anthocyanin extraction

Bulk extraction was done to establish correlation between anthocyanin concentration and absorbance following the procedure of Olawale (2013). Extraction was conducted in 500 ml round bottom flask equipped with water condenser, thermometer and mechanical agitator at atmospheric pressure. Twenty-five grams of dried, milled, screened sorghum bran was weighed, wrapped in a filter paper and kept in the Soxhlet extractor. Ethanol of 99.8% purity (300 ml) was poured into round bottom flask of Soxhlet apparatus and placed on the heating mantle set at a temperature of 60 ^{o}C. Extraction processes commenced and was refluxed till most of the color was extracted from bran sample. After the extraction process, colored extract was concentrated in a rotary evaporator, dried and kept in the desiccator. Calibration curve and equation was generated by preparing different concentrations (through serial dilution) of dried extract with the use of ethanol as the diluent. Absorbances of diluted solutions was determined in UV spectrophotometer in the range 400 - 650 nm. Absorbance data was used for plotting calibration curve (Figure 1) from which optimum wavelength of absorption of the extract was evaluated to be 520 nm.

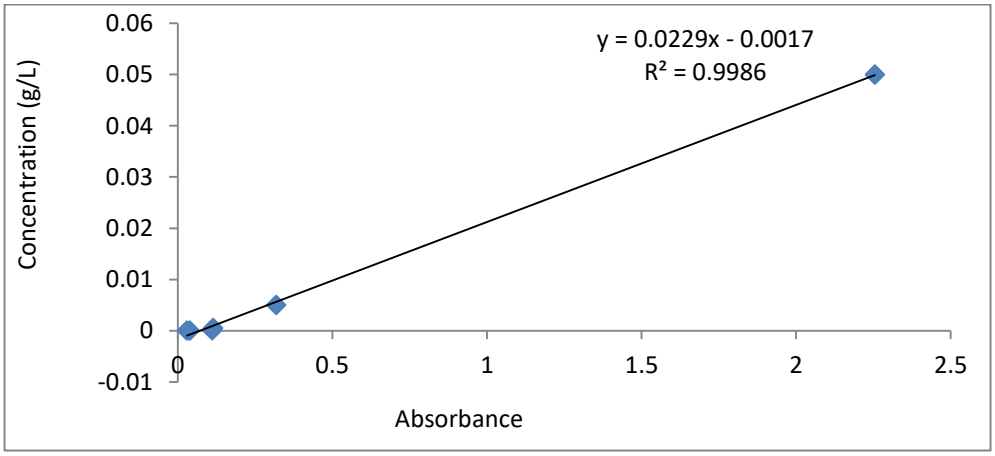


Figure 1: Calibration curve of anthocyanin extract concentrations against absorbances

Subsequent extract concentrations were evaluated using equation 1, and percentage anthocyanin yield evaluated using equation 2 (Zhang and Lui, 2008).

$$C_t = 0.02286\,A - 1.68 \times 10^{-3} \quad (1)$$

$$Y = \frac{Ct*V}{W_o} x100 \quad (2)$$

C_t = concentration of ethanol extract at time t, g/L; A = absorbance of extract from each run; V = volume of solvent used, liters; W_o = Weight of sorghum bran used for each run, g.

Anthocyanin Characterization

Extracted anthocyanin sample was subjected to Fourier Transform Infrared (FTIR) analysis for wavelength number, functional groups and molecular specie of the extract determination at spectra range (1000 - 4000 cm^{-1}).

Experimental design and process optimization

Response Surface methodology (RSM) was used for the study of effects of extraction factors (tabulated in Table 1); solvent volume (A), extraction temperature (B) and extraction time (C) on anthocyanin concentration and yield were studied using one-factor-at-a-time approach and regression model development using Box-Behnken design (BBD). The selection of factors tabulated in Table 1 are modification to existing procedures used for extraction of dietary fibre from sorghum bran (Miafo et al. 2015).

Table 1: Process variables and levels

Factor	Factor name	Units	Actual values		Coded values	
			Low	High	Low	High
A	Solvent	ml	30	70	-1	1
B	Temperature	^oC	40	75	-1	1
C	Time	min	20	60	-1	1

Extraction factors and levels input to BBD generated 17 experimental matrices at specified solvent volume, extraction temperature and time. Anthocyanin concentration and yield were selected responses. Suitable model terms were captured in the BBD model for prediction of anthocyanin concentration and

yield. Individual effects, interactive effects and surface plots were used to analyze influence of extraction factors on extract concentration and yield. Model developed was subjected to optimization studies to determine optimum conditions of the factors (tabulated in Table 1) that maximize anthocyanin concentration and yield. The objective functions are anthocyanin concentration and yield while the constraints are the factors tabulated iin Table 1. Obtained optimal conditions were validated in the laboratory to study percentage deviation of predicted to experimental data using equation 3.

$$e_r = \frac{P_r - E_x}{P_r} * 100 \quad (3)$$

where e_r is the percentage deviation, P_r is the predicted optimal values and E_x is experimental values from the laboratory based on test conditions predicted (P_r).

Results and Discussion

Result of Optimization studies of Anthocyanin Extraction

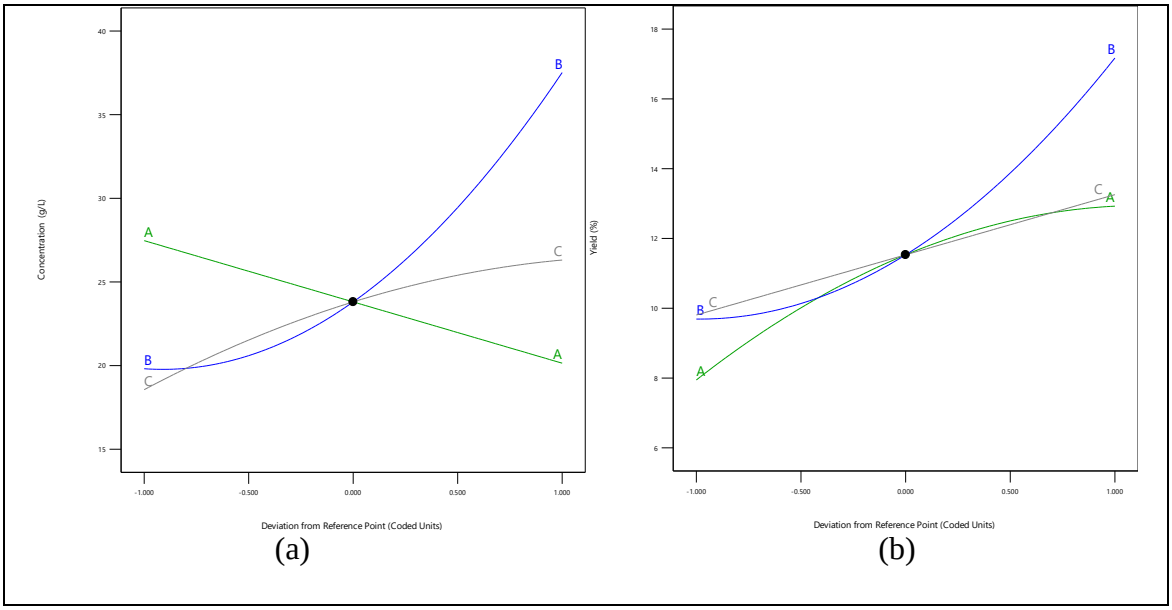
The result of the experimental design using test matrix in Table 1 was presented in Table 2 where the responses from the experimental runs are concentration and yield of anthocyanin respectively. One factor at a time, 3D surface plot, empirical model developed and its validation and optimization condition that favour extraction of anthocyanin were discussed in the subsections below.

One-factor-at-a-time

The individual influence of operating factors; solvent volume (A), temperature (B) and Time (C) on anthocyanin concentration and yield from sorghum bran were presented in Figure 2. Reference point (0) is the mid-point, lowest point (-1) is minimum value and highest point (+1) is the maximum values of the three variables used for this experimental study. For variable A, Concentration decreased with increase in the value of A from 30 to 70 ml (Figure 2a) while increase in the value of A increased the yield of anthocyanin (Figure 2b). Increase in temperature and time within the experimental conditions led to increased anthocyanin concentration while increase in solvent volume decreased it (Figure 2a). Of the three factors, temperature showed the highest influence on anthocyanin concentration. For anthocyanin yield, all the factors contributed positively on individual scale (Figure 2b).

Table 2 : Experimental Design data for anthocyanin extraction from red sorghum bran

Std	Run	Factor1 A: solvent (ml)	Factor 2 B: Temp (^oC)	Factor 3 C: Time (Min)	Response 1 Concentration (g/L)	Response 2 Yield (%)
17	1	50.00	40.00	60.00	24.929	12.46
13	2	50.00	57.50	40.00	23.466	11.73
2	3	70.00	57.50	60.00	20.1513	14.11
8	4	50.00	57.50	40.00	23.466	11.73
16	5	50.00	57.50	40.00	23.466	11.73
9	6	70.00	40.00	40.00	16.0365	11.23
4	7	30.00	57.50	20.00	21.3629	6.42
14	8	30.00	75.00	40.00	38.3936	11.52
10	9	70.00	75.00	40.00	37.4792	19.24
12	10	50.00	40.00	20.00	11.0759	5.54
1	11	50.00	75.00	60.00	35.5361	17.77
3	12	50.00	57.50	40.00	23.466	11.73
15	13	50.00	57.50	40.00	23.466	11.73
7	14	30.00	57.50	60.00	34.3474	10.30
11	15	30.00	40.00	40.00	24.4718	7.34
6	16	50.00	75.00	20.00	35.9247	17.96
5	17	70.00	57.50	20.00	15.6022	10.92



(a) (b)

Figure 2. Effect of individual factors on anthocyanin concentration (a) and yield (b)

Result of Optimization studies

Box Behnken of the Response Surface Methodology (RSM) in the Design Expert 13.0 version was used to generate the models for the two responses based on the result in Table 2. The model that fit the experimental result was quadratic model presented in coded form for both the anthocyanin concentration and yield were presented in equation 3 and 4:

$$Y_1 = +24.5915 - 0.281175A - 1.17997B + 1.31659C + 0.01586B^2 - 3.53613 \times 10^{-3}C^2 + 0.005372AB - 0.005272AC - 0.01017\text{BC} \quad (3)$$

$$Y_2 = -1.45948 + 0.25856A - 0.433003B + 0.39983C - 0.00274A^2 + 0.006201B^2 + 0.002736AB - 0.000431AC - 0.005079BC \quad (4)$$

Experimental design yielded 17 experimental runs and two responses (extract concentration and percentage yield). The 3D surface plots of the three factors considered on extract concentration and yield are presented in Figure 3 and 4 respectively.

In Figure 3a, it was observed that at low extraction temperature of 40 ^{o}C, colourant concentration decreased with increase in solvent and at high extraction temperature of 75 ^{o}C, volume of solvent increased from 30 to 70 ml which led to reduction in colourant concentration from 39.30 to 35.74 g/l. It was also observed that at low solvent volume of 30 ml, increase in temperature from 40 to 75 ^{o}C lead to a corresponding increase in colourant concentration from 25.36 to 39.30 g/l. At high solvent value of 70 ml, there was also an increase in colourant concentration value from 14.27 to 35.74 g/l with increase in temperature. Other extraction concentrations with respect to change in the factors considered are presented in Figure 3b and c respectively.

The 3D surface plot between the simultaneous variation in the values of temperature and volume of solvent used for extraction at mid-point value of time was presented in Figure 4a. At low extraction temperature of 40 ^{o}C, the extraction percentage yield was observed to increase from 7.07 to 10.127 % with increase in solvent volume and at high extraction temperature of 75 ^{o}C, increase in solvent volume from 30 to 70 ml led to an increase in colourant extraction yield from 12.627 to 19.24 %. It was also

observed at low solvent volume of 30 ml that an increase in temperature from 40 to 75 ^{o}C causes the colourant extraction yield to increase from 6.048 to 11.370 %. At high solvent value of 70 ml, there was also an increase in extraction percentage yield value from 9.843 to 14.478 % with increase in temperature. The effect of combination of time and, temperature and time and, volume of solvent used are presented in Figure 3b and c respectively.

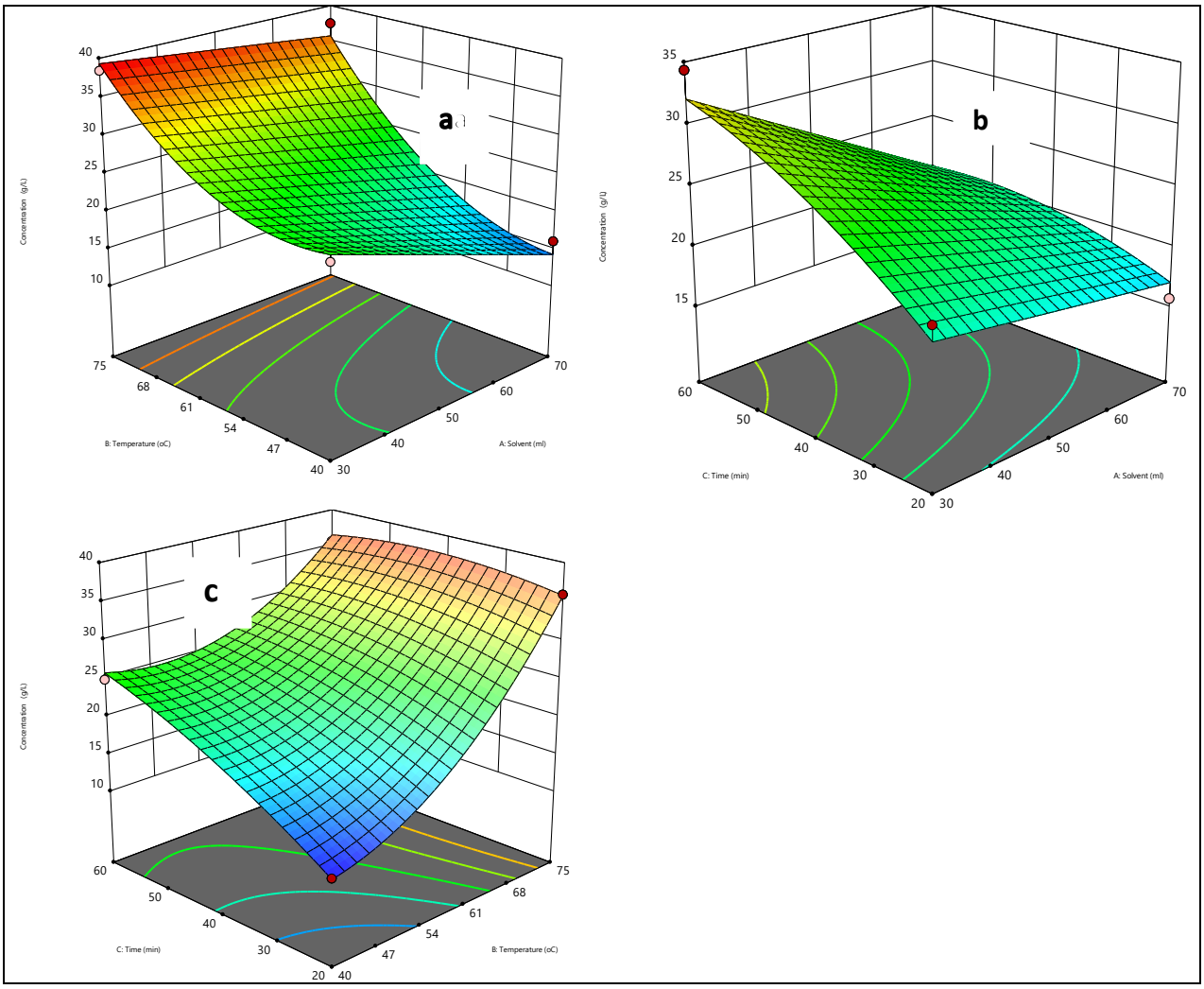


Figure 3: Surface plots of interaction of factors on anthocyanin concentration

Statistical analysis and validation of the model developed

Analysis of variance (ANOVA) was employed to evaluate the significance of the models and the model terms. Both models showed high F values of 55.04 and 40.11 with a very small probability value (p value = 0.0001; 0.0003) for anthocyanin concentration and yield respectively which indicated that the models are significant. The experimental values compared well with predicted values with determination coefficient (R^2) of 0.9822 for anthocyanin concentration and 0.9757 for percentage yield. The adjusted determination coefficient (adj R^2) values for anthocyanin concentration (0.9643) and extraction yield (0.9514) were close to unity suggesting that both models are highly reliable for predicting experimental results.

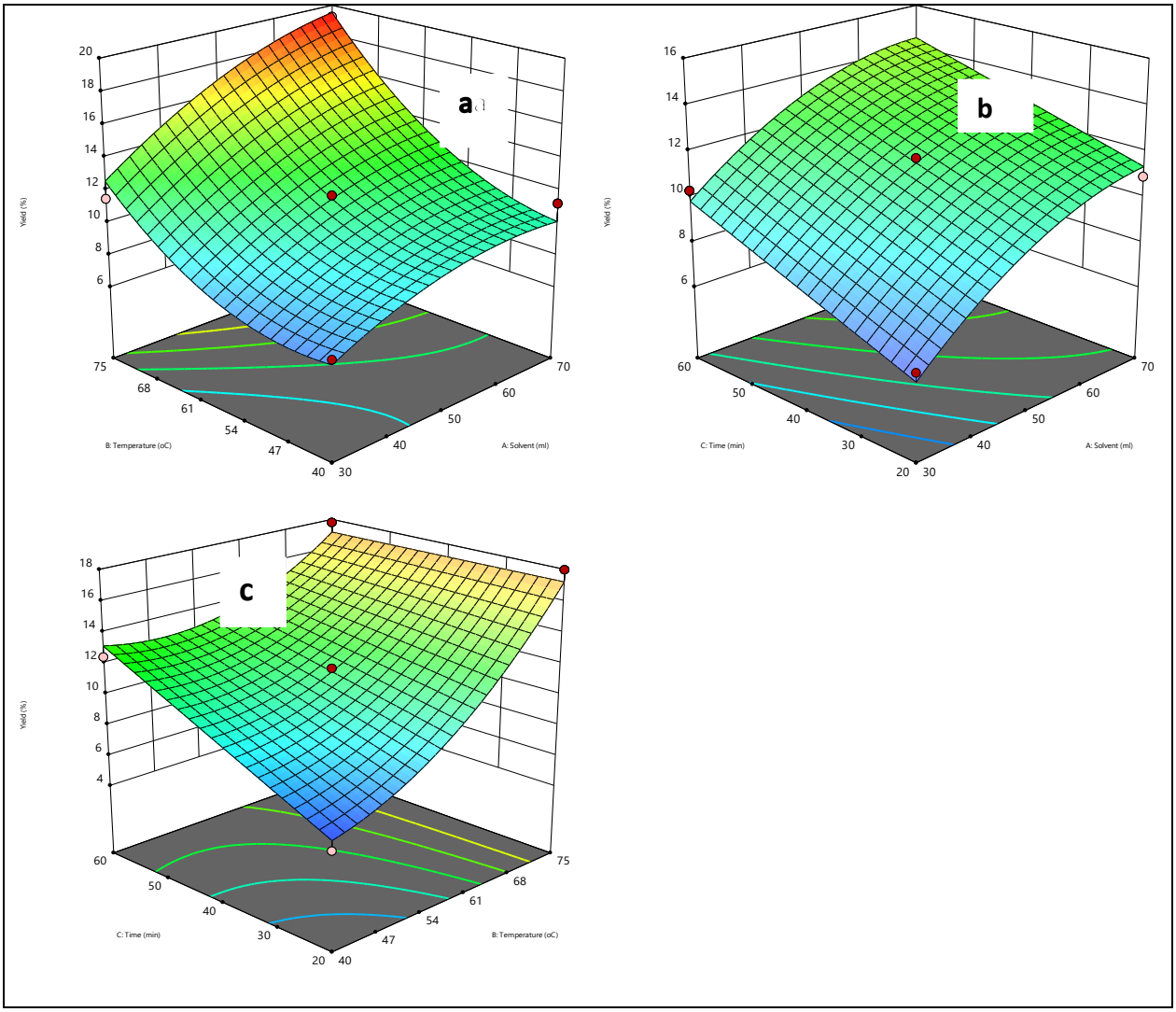


Figure 4: Surface plots of interaction of factors on anthocyanin yield

Optimum anthocyanin concentration (36.469 g/L) and percentage yield (19.2401 %) were obtained at solvent volume (65.1 ml), temperature (75 ^{o}C) and extraction time (30.18 min) with a desirability of 0.969 using ramp of the predicted value (Figure 5a) and 3D-plot of desirability at optimum conditions (Figure 5b).

The experimental values were found to be in good conformity with the predicted values with relatively small deviation (1.4 %; 0.4 %) for concentration and yield respectively (Table 3). The results of validation experiments showed that the model is reliable and in line with the standards established for model validation predictions which prescribed that acceptable minimum error between predicted and experimental data should be less than or equal to 10 % (Oke *et al.*, 2010).

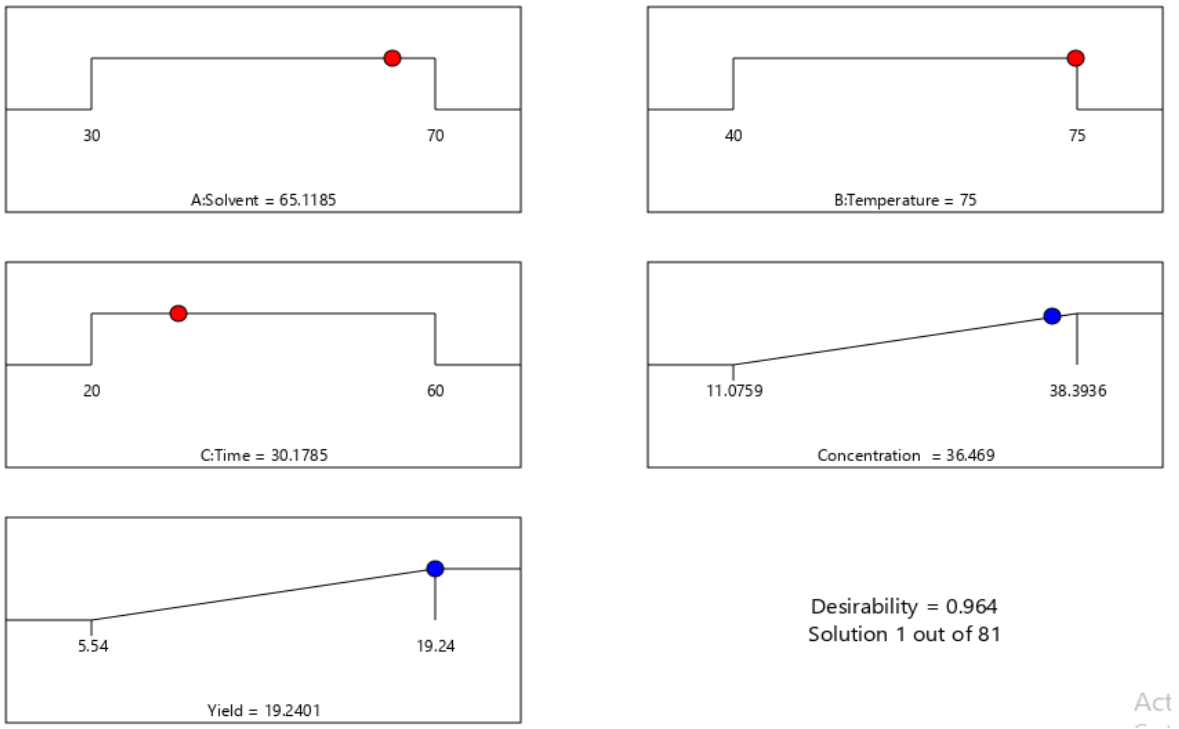


Figure 5a: Optimization results ramp for sorghum bran anthocyanin production.

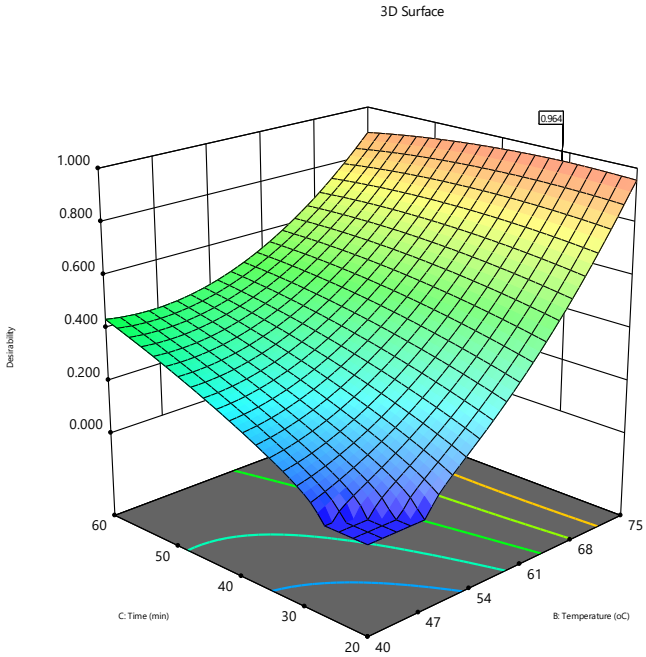


Figure 5b: Plot of Desirability at Optimum Conditions

Table 3: Validation of Optimum process conditions for Athocyanin production

	Predicted results	Experimental results	Standard deviation
Yield (%)	19.2401	19.317	0.4
Concentration (g/L)	36.469	36.980	1.4

Extract characterization

Infra-red (IR) peaks observed from anthocyanin extract at the range 1000 - 4000 cm^{-1} was presented (Figure 6) and the corresponding functional groups (Table 2). Absorption peaks 729.9cm^{-1}, 975.2cm^{-1} and 1916.6cm^{-1} indicated the presence of C=C alkene. Peaks at 822.4cm^{-1} indicated strong and medium C-Cl stretching of halo-compound while the peak at 1123.1cm^{-1} indicated strong C-O stretching of secondary alcohol. The peak at 1709.0cm^{-1} showed presence of C=O carboxylic acid and conjugated acid in the extract. Peaks at 2024.0cm^{-1} indicated strong N=C=S isothiocyanate group while the peaks at 2711.3cm^{-1}, 2898.4cm^{-1}, 3303.0cm^{-1} and 3041.5cm^{-1} indicated the presence of identical C-H stretching group of aldehyde, alkane, alkyne and alkene group respectively. N-H stretching of aliphatic primary amine group was indicated by peak at 3395.9cm^{-1} and O-H group of carboxylic acid at peak 3534.3cm^{-1}. The presence of strong isothiocyanates in the extract indicates anthocyanin property of sorghum bran extract (Sandi *et al.* 2011). Comparing the FTIR spectrum of acidified methanol extract reported by Duodu (2012) with the FTIR spectrum of the extract in this study suggests that chemical composition of extract from red sorghum bran is dependent on the solvent used for extraction.

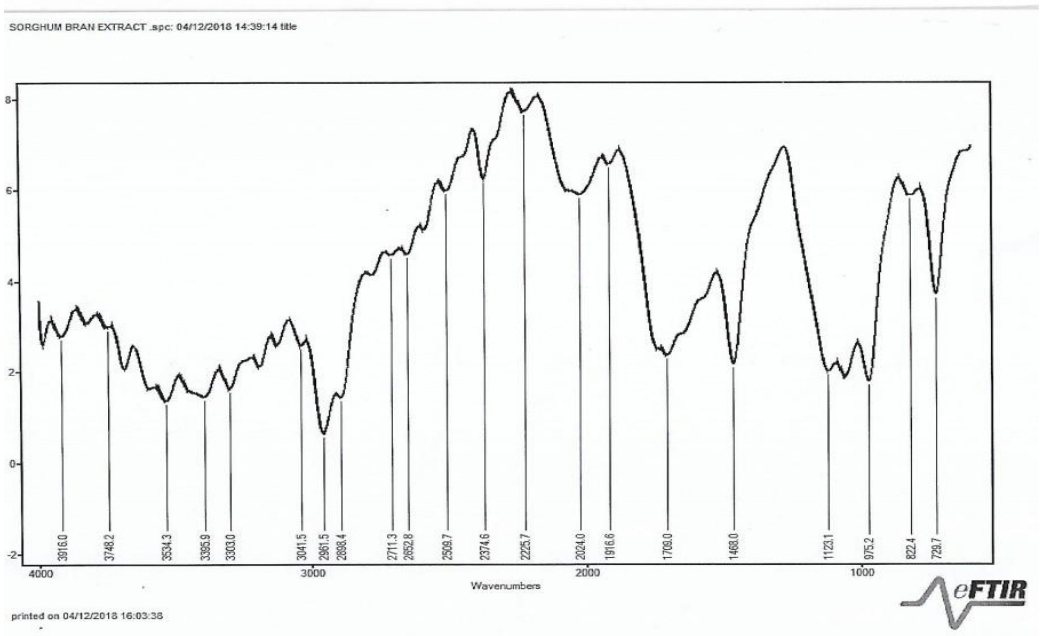


Figure 6: IR bands of anthocyanin extract from FTIR spectroscopy.

Table 4: Infrared Spectroscopy of anthocyanin extract from red sorghum bran

Peak (cm^{-1})	Appearance	Bonding
729.7	Strong	C=C bending (alkene), di-substituted (cis)
822.4	strong, medium	C-Cl stretching (halo-compound)
975.2	strong	C=C bending (alkene)
1123.1	strong	C-O stretching (secondary alcohol)
1709.0	strong	C=O stretching (Carboxylic acid, conjugated acid)
1916.6	medium	C=C=C stretching (alkene)
2024.0	strong	N=C=S Stretching (iso-thiocyanate)
2711.3	medium	C-H stretching (aldehyde)
2898.4	medium	C-H stretching (alkane)
3303.0	strong	C-H stretching (alkyne)
3041.5	medium	C-H stretching (alkene)
3395.9	medium	N-H stretching (aliphatic primary amine)
3534.3	strong	O-H stretching (carboxylic acid)

Result of Kinetic Studies

To evaluate appropriate kinetic model describing the process of anthocyanin extraction from red sorghum bran, experimental data were generated in the laboratory at varied temperatures (50 ^{o}C, 60 ^{o}C and 70 ^{o}C). These equations were linearized and fitted into first order and second order kinetic models respectively.

Linearized First order kinetic equation;

$$\log(C_t) = \log(C_s) - \frac{K_1}{2{,}303}t \quad (5)$$

Linearized Second order kinetic equation;

$$\frac{1}{C_t} = \frac{1}{C_s} + kt \quad (6)$$

In order to determine the rate constant for both first and second order kinetic(k), data obtained for this investigation was fitted with equation 5 and 6 respectively. The outcome of the fitting was tabulated in table 6. This showed that first order kinetic model is most suitably for the describtion of process of anthocyanin extraction from sorghum bran because correlation coefficient (R^2) of first order kinetic are greater than thoat of secdonrd order kinetic fitting regardless of the temperature used.

Table 6: Linearization of First and Second Order Kinetic Models

Temperature	Calculation method	Slope	K_1	Intercept	R^2
First order model					
		0.0106	0.0244	1.3803	0.786
70 ^{o}C	Linear Regression				
60 ^{o}C	Linear Regression	0.0114	0.0263	1.4692	0.746
50 ^{o}C	**Linear Regression**	**0.0106**	**0.0244**	**1.3803**	**0.789**
Second order model					
70 ^{o}C	Linear Regression	0.3127	0.3127	26.756	0.4537
60 ^{o}C	Linear Regression	0.4099	0.4099	34.158	0.4912
50 ^{o}C	Linear Regression	0.4788	0.4788	39.692	0.5081

Conclusion

This study has established the possibility of anthocyanin extraction from red sorghum bran using ethanol as solvent. Application of response surface methodology showed that process parameters like solvent volume, extraction temperature and extraction time have significant effect on anthocyanin concentration and yield from sorghum bran. Regression model based on BBD of RSM conforms well to experimental results for both anthocyanin concentration and yield. Optimum model efficiency (96.4%) based on the regression model was obtained at solvent volume; 65.1 ml, extraction temperature; 75 ^{o}C and extraction time of 30.18 minutes. Results of kinetic study showed that kinetic data for anthocyanin extraction from red sorghum bran exhibits a time dependent behavior which conform to first order kinetic model with very high coefficient of determination (R^2). The optimum operating conditions established in the laboratory could be adopted for large scale design of anthocyanin production from red sorghum bran. The process is environmentally benign, a sustainable means of anthocyanin production from cheap, readily available crop material usually regarded as waste and an eco-friendly means of wastes valorization.

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