OPTIMIZATION AND RESPONSE SURFACE MODELLING OF ANTIOXIDANT ACTIVITIES OF *Amaranthus virides* SEED FLOUR EXTRACT

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Abstract
Plants from the Amaranth genus are attracting researchers' attention mainly because of their high nutritional value and nutraceutical properties. Various organic solvents such as acetone, ether, ethanol and methanol are used to obtain seed extracts. However, to extracts antioxidant from plant seed, a combinations of process variables need to be optimized to increase the process efficiency. In this work, response surface methodology (RSM) was used to determine the optimum condition for the extraction of antioxidant compounds from Amaranthus virides seed flour. The Box-Behnken design was used to examine the effect of three process variables, methanol concentration (80%, 90% and 100%), extraction temperature (40, 50 and 60 ˚C) and extraction time (30, 45 and 60 min). Second-order polynomial model was used for predicting the response. The results showed that total phenolic content (TPC), total flavonoid content (TFC) and DPPH antioxidant activity in the experiments varied from 0.51 to 8.35 mg GAE/100mg, 10.01 to 24.07 mg QE/g and 34.68% to 97.23%, respectively. Under the optimum conditions of 80% methanol, 42.65 ˚C extraction temperature and extraction time of 30.09 min, the values for the total phenolic content, total flavonoid content and DPPH antioxidant activity were 0.8174 mg gallic acid equivalent GAE/100mg, 20.466 mg quecetin equivalent QE/g and 97.23% respectively. The experimental values are in accordance with those predicted, indicating the suitability of the employed model and the success of RSM in optimizing the extraction conditions.

Keywords: Amaranthus virides, Antioxidant activity, Response surface, Extraction Optimization, Total Flavonoid content, Total Phenolic content

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1. INTRODUCTION

Lipid oxidation can lead to the deterioration of food quality, shorten the shelf-life of food products and reduce the acceptability of processed foods (Zhidong *et al*., 2013). This can however generate free radicals which can also readily react with and oxidizes most biomolecules, including carbohydrates, proteins, lipids, DNA and small cellular molecules (Peng *et al.,* 2009). Free radicals are believed to play a significant role in the occurrence of diseases, such as cardiovascular diseases, diabetes mellitus, neurological disorders and Alzheimer's disease (Stadtman, 2006). It is therefore necessary to retard lipid oxidation and the formation of free radicals in food and biological systems (Moskovitz *et al.*, 2002). Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), n-propyl gallate (PG), and t-butylhydroquinone are commonly used to retard lipid peroxidation and to curtail the formation of free radicals in food and biological systems (Peng *et al*. 2009). However, the application of synthetic antioxidants is restricted due to potential risks related to human health (Hraš *et al*. 2000). Therefore, in order to inhibit lipid peroxidation in food products and enhance the body's antioxidant defense, interest has been developed in identifying natural antioxidants.
Plants from the Amaranth genus are attracting researchers' attention mainly because of their high nutritional value. Due to their high nutritive and nutraceutical characteristics, they have excellent agronomic features (Breene, 1991; Saunders *et al*., 1984; Barba *et al*., 2009; Acevedo *et al*., 2007; Bressani, 2003). Amaranthus virides is a broad-leaf pseudo-cereal with an upright growth habit, cultivated for both its seeds which are used as grain and its leaves used as vegetable (Kadiri and Olawoye, 2015). Amaranthus virides seed have remarkable nutritional composition, not only from its protein content (13-14%), but also

from its high amino acid balance. But, beyond their nutritional function of supplying nutrients, *Amaranthus virides* seeds provide compounds with promoting health properties such as phenolic acids, phytosterols and flavonoids (Abugoch, 2009).

Extraction is the first step in isolation of antioxidants from plant material and plays a crucial stage in quantification and identification of these compounds. Many factors including type of solvent, the temperature, the pH, the number of extraction steps, liquid-to-solid ratio and the particle size of the solute contribute to the efficacy of the extraction process (Mafart and Beliard, 1992). compounds. Optimization of antioxidants extraction may be achieved by either empirical or statistical methods and is essential for commercial application of the bioactive compounds extraction process (Rodrigues *et al*., 2008; Annegowda *et al*., 2012). Response surface methodology (RSM) is a statistical experimental protocol used in mathematical modeling (Triveni *et al*., 2001; Gong *et al*., 2012). This method reduces the experimental assays thus improving the statistical interpretation, possibility and interaction between variables (Tsapatsaris *et al*., 2004; Yim *et al*., 2012). Using statistical software, for example JMP (SAS Institute Inc.), the RSM can give a mathematical equation. Moreover, it is helpful to calculate the response value when different levels of variables are set. Box–Behnken design is a widely used protocol in response surface methodology (Yang *et al*., 2008; Rao, 2010). In order to study the influence of three parameters that affected the extraction of antioxidants from *Amaranthus virides* seed flour, we fixed as objective in this investigation the optimization of extraction conditions (solvent concentration, temperature, and time) of total phenolic contents, total flavonoid content, total antioxidant content and antioxidant activity from *Amaranthus virides* seed four using RSM methodology.

2. MATERIALS AND METHODS

Sun dried Amaranthus virides seeds, harvested from the South-west town of Nigeria (Ondo), was used in this study. The identity of the seed was verified and confirmed at the herbarium section of the Department of Botany, Obafemi Awolowo University Ile-Ife, Nigeria. The amaranth seeds were converted to flour using an electric grinder (Kenwood model, UK)), packaged in polyethylene bags and stored at 4 ˚C in a refrigerator prior to extraction and analysis. The chemicals used in this research were purchased from Sigma chemicals, USA and were of analytical grade.

2.1. Extraction process

Table 1. Coded and decoded levels of independent variables used in the RSM design.

Run	Variable levels		
	X1	X2	X3
1	100 (+1)	50 (0)	60 (+1)
2	100 (+1)	40 (-1)	45 (0)
3	80 (-1)	50 (0)	30 (-1)
4	80 (-1)	60 (+1)	45 (0)
5	80 (-1)	50 (0)	60 (+1)
6	90 (0)	60 (+1)	30 (-1)
7	100 (+1)	50 (0)	30 (-1)
8	90 (0)	40 (-1)	60 (+1)
9	80 (-1)	40 (-1)	45 (0)
10	100 (+1)	60 (+1)	45 (0)
11	90 (0)	50 (0)	45 (0)
12	90 (0)	40 (-1)	30 (-1)
13	90 (0)	50 (0)	45 (0)
14	90 (0)	60 (+1)	60 (+1)
15	90 (0)	50 (0)	45 (0)

X_1, solvent concentration (%); X_2, Extraction time (min); X_3, Extraction temperature (˚C)

Dried Amaranthus virides seed flour (2.5g) were placed in a 250mL conical flask with 100mL of solvent containing variable amounts of methanol/water. Extractions were carried out under magnetic stirring at 400 rpm, at different temperature and time (Table 1). The extract was filtered through Whatman No. 4 filter paper and then centrifuged at 10,000 rpm (BOSH Centrifuge TDL-5) for 10 min.

2.2. Total Phenolic Content (TPC)

The total phenolic content was determined according to the Folin-Ciocalteu method (Kähkönen *et al.* 1999). Briefly, 0.2 mL of the filtrate was added to a 25 mL volumetric flask, and additional distilled water was added to make a final volume of 10 mL. A reagent blank was prepared using distilled water. Folin-Ciocalteu phenol reagent (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 mL of 5 % Na_2CO_3 solution was added with mixing. The solution was immediately diluted to 25 mL with distilled water and mixed thoroughly and then allowed to stand for 90 min. After that, the absorbance was measured at 750nm versus the prepared blank. The total phenolic yield of the sample was expressed as gallic acid equivalents (GAE) milligrams/g raw material.

2.3. Total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination (Wang and Jiao, 2000). 1 ml of the plant extracts/standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min then absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following equation:

$$C = (c \times V)/m \quad (1)$$

Where; C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent, c = the concentration of quercetin established from the calibration curve in mg/ml, V = the volume of extract in ml and m = the weight of crude plant extract in gm.

2.4. Antioxidant activity (DPPH)

The determination was carried out as described by Pownall *et al.*(2010). Aliquots (50 µL) of extracts were added to 1950 µL of a methanolic solution (100 µM) of DPPH radical. After agitation, the mixture was incubated in the dark for 30 min and the absorbance was measured at 517 nm in an UV 1240 spectrophotometer (Shimadzu, France). Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract but the methanolic solution (100 µM) of DPPH radical and absorbance read at 517 nm using a spectrophotometer. Methanol served as the blank.

$$Inhibition(\%) = \frac{(Acontrol - Asample)}{Acontrol} \times 100 \quad (2)$$

2.5. Total antioxidant activity

The total antioxidant activity was eluted by using the method described by Prieto *et al* (1999). Plant extracts were dissolved in methanol to obtain a concentration of 500 µg/ml. 3 ml of extract was placed in a test tube, 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate, 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 95˚C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of each solution was measured by using UV-Visible spectrophotometer at 695 nm against blank. A calibration curve was constructed, using ascorbic acid (100-500 µg/ml) as standard and total antioxidant activity of extract (µg/ml) expressed as ascorbic acid equivalents.

2.6. Experimental design

The extraction process for the *Amaranthus virides* seed flour extract was established by RSM which was employed to determine the best combination of variables for optimum total phenolic content, DPPH radical scavenging

activity, total flavonoid and antioxidant activity. The independent variables used in this study were methanol concentration (x_1, % v/v), extraction temperature (x_2, ˚C), and time (x_3, min) while response variables were TPC, DPPH, TFC and TAC. Coded and uncoded levels of the independent variables and the experimental design were presented in Table 1 and 2. Coded value 0 stands for center point of the variables and was repeated for experimental error. Factorial points were coded as ± 1**2.7.**

Statistical analysis

The response surface regression procedure of JMP 11 (statistical analysis system Inc., SAS) was used to analyze the experimental data. P-values below 0.05 were regarded as significant. The quality of the mathematical models fitted by RSM was evaluated by ANOVA, based on the *F*-test and on the percentage of total explained variance (R) and also on the adjusted determination coefficient (R^2adj), which provide a measurement of how much of the variability in the observed response values could be explained by the experimental factors and their linear and quadratic interactions (Granato *et al*. 2010b) A second-order polynomial quadratic equation (Equation 2) was used to fit the results:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum \sum_{i<j} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \epsilon. \quad (3)$$

Where: Y is the predicted response, β_0, β_i, β_{ii}, β_{ij}, are the correlation coefficients for intercept, linear, quadratic and interaction terms, respectively and x_i and x_j are the levels of the independent variables. Experimental data were then fitted to the selected regression model to achieve a proper understanding of the correlation between each factor and different responses. Optimal conditions for extraction of total phenolic content, total flavonoid content, total antioxidant activity and DPPH radical scavenging activity from Amaranthus virides seed flour depended on solvent concentration, extraction temperature and extraction time were obtained using the predictive equation of RSM. The experimental and predicted values of the responses were compared in order to determine the validity of the model.

Table 2. Experimental and Predicted Result for the Antioxidants

Run	TPC (mg GAE/100mg)		TFC (mg QE/g)		DPPH (% inhibition)	
	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	0.51	0.03	21.83	23.18	36.21	33.88
2	1.17	1.78	14.05	13.71	47.72	46.81
3	6.47	6.96	24.02	22.67	85.17	87.5
4	8.35	7.74	10.12	10.46	88.27	89.18
5	5.08	5.66	12.24	13.30	92.24	86.82
6	1.25	1.37	10.03	11.04	55.60	52.36
7	1.05	0.47	19.64	18.58	34.68	40.10
8	0.72	0.59	10.01	9.00	53.45	56.69
9	7.99	7.53	10.03	9.98	97.23	99.41
10	0.91	1.38	12.47	12.52	43.61	41.44
11	3.75	3.88	10.34	10.21	42.73	42.91
12	4.91	4.88	10.23	11.64	57.56	53.05
13	3.99	3.89	10.01	10.21	41.07	42.91
14	3.89	3.91	10.30	8.89	37.28	41.79
15	3.92	3.88	10.28	10.21	44.95	42.91

Table 3. Regression Coefficient, Standard Error, and Student's T-Test Results of Response Surface for TPC, TFC and DPPH antioxidant activity

Term	Estimate	Std Error	t Ratio	Prob>|t|
TPC				
Intercept	3.885	0.398	9.75	0.0002*
Solvent Concentration (X_1)	-3.030	0.244	-12.43	<.0001*
Extraction Temperature (X_2)	-0.049	0.244	-0.20	0.8480
Extraction Time (X_3)	-0.436	0.244	-1.79	0.0505
X_1X_2	-0.153	0.345	-0.44	0.6763
X_1X_3	0.214	0.345	0.62	0.5623
X_2X_3	1.707	0.345	4.95	0.0002*
X_1^2	0.655	0.359	1.83	0.0043*
X_2^2	0.067	0.359	0.19	0.8601
X_3^2	-1.260	0.359	-3.51	0.0171*
TFC				
Intercept	10.210	0.899	11.35	<.0001*
Solvent Concentration (X_1)	1.447	0.551	2.63	0.0467*
Extraction Temperature (X_2)	-0.176	0.551	-0.32	0.7623
Extraction Time (X_3)	-1.192	0.551	-2.16	0.0301*
X_1X_2	-0.419	0.779	-0.54	0.6138
X_1X_3	3.491	0.779	4.48	0.0065*
X_2X_3	0.123	0.779	0.16	0.8810
X_1^2	5.373	0.811	6.63	0.0012*
X_2^2	-3.915	0.811	-4.83	0.0048*
X_3^2	3.848	0.811	4.75	0.0051*
DPPH				
Intercept	42.914	3.164	13.56	<.0001*
Solvent Concentration (X_1)	25.086	1.937	-12.95	<.0001*
Extraction Temperature (X_2)	3.898	1.937	-2.01	0.014*
Extraction Time (X_3)	1.728	1.937	-0.89	0.0413*
X_1X_2	1.212	2.739	0.44	0.6766
X_1X_3	-1.384	2.739	-0.51	0.6348
X_2X_3	-3.552	2.739	-1.30	0.2514
X_1^2	18.697	2.852	6.56	0.0012*
X_2^2	7.597	2.852	2.66	0.0447*
X_3^2	0.462	2.852	0.16	0.8777

*Values statistically significant at $p < 0.05$

3. RESULT AND DISCUSSION

3.1. Analysis of the Model

The result for the analysis of the model for the antioxidants optimization are listed in Table 3. The regression coefficient of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique as shown in Table 3.

In this result, X1, X3, X2X3, $X1^2$ and $X3^2$ were found to be significant at the level of $p<0.05$ for TPC, whereas X1, X3, X1X2, X1, X2 and X3 were significant at the level of ($p<0.005$) for TFC.

Three linear (X1, X2, X3), and two quadratic parameters (X1 X2) were found to be significant at the level of ($p<0.05$) for DPPH. The fitted quadratic model for TPC, TFC and DPPH were showing equation 2,3 and 4.

$$TPC = 3.885 - 3.030X_1 - 0.049X_2 - 0.436X_3 - 0.153X_1X_2 + 0.214X_1X_3 + 1.707X_2X_3 + 0.655X_1^2 + 0.067X_2^2 - 1.260X_3^2 \quad (4)$$

$$TFC = 10.210 + 1.447X_1 - 0.176X_2 - 1.192X_3 - 0.419X_1X_2 + 3.491X_1X_3 + 0.123X_2X_3 - 5.373X_1^2 - 3.915X_2^2 + 3.848X_3^2 \quad (5)$$

$$DPPH = 42.914 + 25.086X_1 + 3.898X_2 + 1.728X_3 + 1.212X_1X_2 - 1.384X_1X_3 - 3.552X_2X_3 + 18.697X_1^2 + 7.597X_2^2 + 0.462X_3^2 \quad (6)$$

Table 4. Analysis of Variance (ANOVA) for Quadratic Model

Responses	Source of variation	Sum of Square	DF	Mean Square	F Ratio	Prob>F
TPC	Model	94.93	9	10.547	22.169	0.0016
	Error	2.378	5	0.476		
	Lack of fit	2.347	3	0.7823	48.448	0.0203
	Pure error	0.032	2	0.016		
	Cor. Total	97.309	14			
R2 = 0.9755, R (adjusted) = 0.9315						
TFC	Model	306.356	9	34.039	14.023	0.0048
	Error	12.136	5	2.427		
	Lack of fit	12.073	3	4.024	128.011	0.0078
	Pure error	0.063	2	0.0314		
	Cor. Total	318.493	14			
R2 = 0.9619, R (adjusted) = 0.900						
TAC	Model	148.553	9	16.506	10.178	0.01
	Error	8.108	5	1.622		
	Lack of fit	5.291	3	1.764	1.252	0.4728
	Pure error	2.817	2	1.408		
	Cor. Total	156.661	14			
R2 = 0.9411, R (adjusted)= 0.9081						
DPPH	Model	6683.092	9	742.566	24.729	0.0013
	Error	150.136	5	30.027		
	Lack of fit	142.544	3	47.513	12.518	0.0749
	Pure error	7.592	2	3.796		
	Cor. Total	6833.228	14			
R2 = 0.9780, R (adjusted) = 0.9385						

From the equations above, it is evident in equation 4 that the interaction parameter (X_2X_3) had the highest positive effect on the total phenolic content while the solvent concentration (X_1) had the highest negative effect on the total phenolic content. Also from equation 5, the quadratic term (X_1^2) had the highest positive effect on total flavonoid content while the quadratic term (X_2^2) had the highest negative effect on total flavonoid content. Solvent concentration had the highest positive effect as shown in equation 5 while the interaction term (X_2X_3) had the highest negative effect on DPPH. Table 4 show the results of fitting quadratic model of the data. The result of the analysis of variance (ANOVA) indicate that the model was significant ($p<0.05$) for response of the dependent variables (TPC, TFC and DPPH radical scavenging activity). The result of the Anova also indicate a good model performance with correlation coefficient (R^2) values of 0.9755, 0.9619 and 0.9780 for TPC, TFC and DDPH respectively. These explain 97.55, 96.19 and 97.8% of calculated model. The p value of 0.0016, 0.0048 and 0.0013 for TPC, TFC and DDPH indicated that the statistical analysis is of high significant level, attesting

the goodness of fit for the optimized antioxidants. This result indicated that the statistical model could work well for the prediction of the studied antioxidant from *Amaranthus virides* seed flour.

3.2. Analysis of response surface

The best way of expressing the relationship between the independent variables and dependent variables (TPC, TFC and DPPH) is to graphically plot the response surface plots generated by the model (Fig. 1-3).

Figure 1a showed the interaction between solvent concentration and extraction temperature while holding the extraction time at the centre point (0). The result showed that high temperature up to 60˚C and low solvent concentration favour high extraction of TPC, while increasing both led to low value of total phenolic content. Many researchers had shown that increasing solvent concentration up to 80% increased the yield of polyphenol and antioxidant activity. This was attributed to moderately polarity of the solvent. Liyana-pathirana and Shaludi (2005) reported an increase in TPC value with increasing temperature up to 60˚C followed by a decline in TPC values with increase in temperature for all wheat materials. This they attributed to thermal degradation of these compounds at higher temperature. Fig. 1b showed the interaction effect of extraction time and temperature and their reciprocal interaction on TPC while keeping solvent concentration constant. The result showed an increased extraction time coupled with increase in extraction temperature favoured TPC value while decreasing both factor lead to low TPC value.in Fig 1c, the TPC value was favoured by an increase in extraction time with a decrease in solvent concentration. Solvent concentration above 80% led to gradual reduction in TPC value.

Fig.2a-c showed the effect of the independent variables and their interactions on DPPH radical scavenging activities. The result (fig 2a) showed an increase in extraction temperature coupled with reduced solvent concentration increased in DPPH radical scavenging activity. The solvent concentration alone had more pronounced effect on DPPH than the extraction temperature as seen in Table 3. On the interaction of extraction time and temperature on DPPH while the solvent concentration is held constant as shown in fig. 2b, increase in both extraction temperature and time had negative effect on the DPPH radical scavenging activities. Both the extraction time and temperature displayed both linear and quadratic effect on the DPPH radical scavenging activity. On the effect of extraction time and solvent concentration as shown in fig 2c, interaction between extraction time and solvent temperature had negative effect on the DPPH radical scavenging activities. However, both the extraction time and solvent concentration showed both linear and quadratic effect on DPPH radical scavenging activities.

Fig 3a-c show the effect and interaction of the independent variables on the total flavonoid content of the *Amaranthus virides* seed flour extract. As shown in figure 3a, when the extraction time was fixed at the centre point (0), the interaction of the solvent concentration and extraction temperature had an insignificant effect on the total flavonoid content while the solvent concentration displayed linear and quadratic effect on the TPC. In figure 3b, when the extraction temperature is held at the centre point, the result shows that increase in extraction time coupled with increase in solvent concentration increase the total flavonoid yield. Also in figure 3c, when the solvent concentration was held constant at the centre point, the extraction time had the major effect on the TFC value than the extraction temperature. Increase in extraction temperature from 48 – 59˚C coupled with increased extraction time increase the total flavonoid (TFC) yield. Also the extraction temperature had insignificant effect on the TFC yield. **3.3. Process Optimization**

The optimal value of the independent variables for the extraction of TPC, TFC, and DDPH radical scavenging activity were determined using the maximum desirability. Result of optimal conditions to obtain the highest extraction phenolic and flavonoid from

Amaranthus virides seed flour as well as maximizing DPPH radical scavenging activity were 80% solvent concentration, 42.65˚C extraction temperature and extraction time of 30.09 mins, at which the values for the total phenolic content, total flavonoid content and DPPH radical scavenging activity were 0.8174 mgGAE/100mg, 20.466 mg QE/g and 93.89% inhibition respectively. Therefore, the regression equations obtained in this study can be used to draw extract from *Amaranthus virides* seed flour with optimum total polyphenolic content, total flavonoid content and DPPH antioxidant activity.

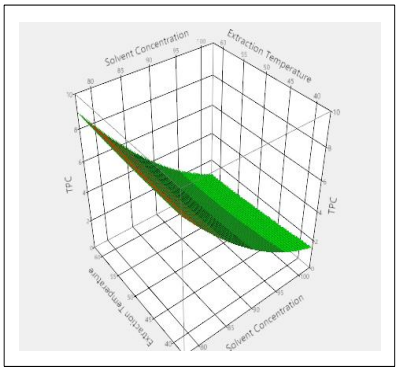

Fig. 1a

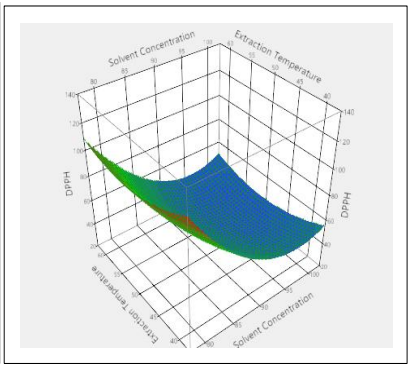

Fig. 2a

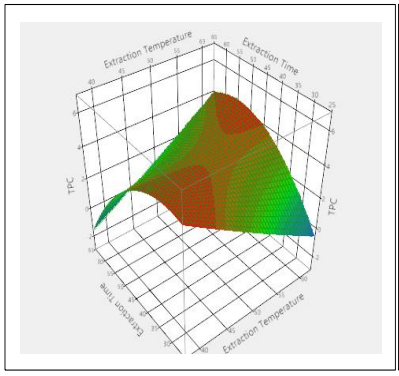

Fig. 1b

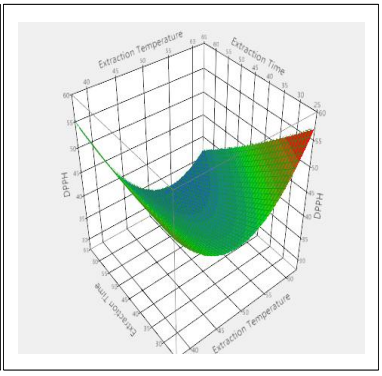

Fig. 2b

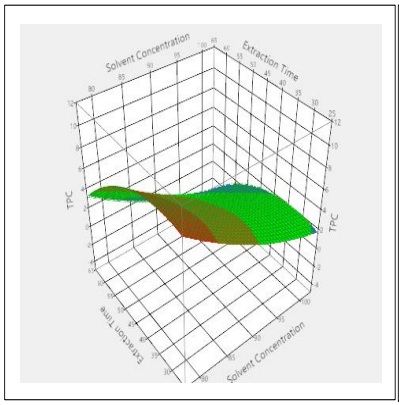

Fig. 1c

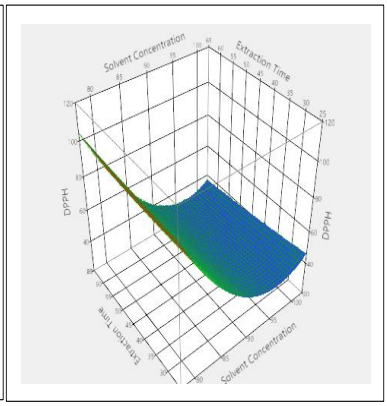

Fig. 2c

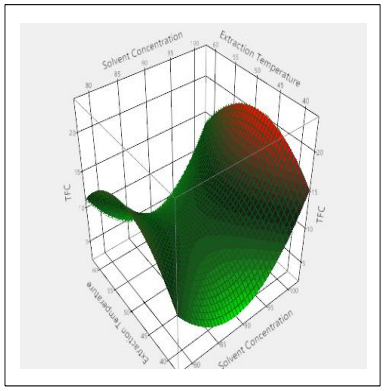

Fig. 3a

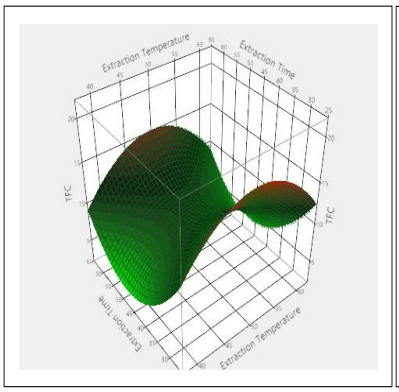

Fig. 3b

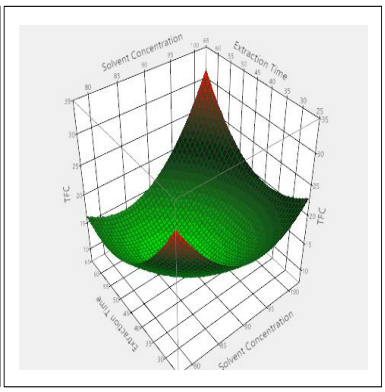

Fig. 3c

Figure 1-3. Response surface plots showing the effects of (a) extraction temperature (˚C) and solvent concentration (%), (b) extraction time (min) and extraction temperature (˚C), (C) extraction time (min) and solvent concentration (%) on TPC, DPPH radical scavenging activities and TFC respectively.

4. CONCLUSIONS

The methanolic extracts of *Amaranthus virides* seed flour was analyzed for its total phenolic contents, total flavonoid contents and its corresponding DPPH antioxidant capacity following fifteen different combinations of three independent variables, viz. methanol concentration, extraction temperature and extraction time as per the experimental design. High correlation of the mathematical model indicated that a quadratic polynomial model may be employed to optimize the solid-liquid extraction of antioxidants from *Amaranthus virides* seed flour. From the response surface plots, all the three studied independent variables (methanol concentration, extraction temperature and extraction time) significantly influenced TPC, TFC and DPPH antioxidant activity of Amaranth extracts. Using the response surface methodology, the optimum condition of methanol concentration, extraction temperature and time was obtained. The optimum conditions were 80% methanol concentration, 42.65 C extraction temperature and 30.09 mins extraction time, at which the value of TPC, TFC and DPPH antioxidant activity were 0.8174 mg GAE/100mg, 20.466 mgQE/g and 93.89 respectively. The results confirm the predictability of the model for the extraction of TPC, TFC and DPPH Antioxidant activity from *Amaranthus virides* seed flour in the experimental conditions used.

5. REFERENCES

[1] Abugoch, L., Romero, N., Tapia, C., Silva, J., and Rivera, M. Study of some physicochemical and functional properties of quinoa (Chenopodium quinoa Willd.) protein isolates. *Journal of Agriculture and Food Chemistry*, 2008, 56, 4745–4750.

[2] Acevedo I, García O, Acevedo I, Perdomo. Nutritional value of amaranth (*Amaranthus* spp) identified in the municipality Moran, Lara State. In: AGROLLANIA, 2007 4: 77-93.

[3] Annegowda, H. V., Mordi, M. N., Ramanathan, S., Hamdan, M. R. and Mansor, S. M. Effect of extraction techniques on phenolic content, antioxidant and antimicrobial activity of *Bauhinia purpurea*: HPTLC determination of antioxidants. *Food Analytical Methods*, 2012, 5: 226–233.

[4] Barba de la Rosa AP, Fomsgaard I, Laursen B, Mortensen A, OlveraMartínez J, Silva C, Mendoza A, De León A,

[5] Breene WM. Food uses of grain amaranth. *Cereal Food World*, 1999, 36: 426-430. Bressani R. Amaranth. In: B. Caballero, Editor, Encyclopedia of food sciences and nutrition, *Academic Press, Oxford*, 2003, pp. 66-173.

[6] Gong, Y., Hou, Z., Gao, Y., Xue, Y., Liu, X. and Liu, G. Optimization of extraction parameters of bioactive components from defatted marigold (*Tagetes erecta* L.) residue using response surface methodology. *Food and Bioproducts Processing*, 2012, 90: 9–16.

[7] Granato, M., Zekovic´ , Z., Jokic´ b, S., Vidovic´ , S. Determination of optimal extraction parameters of mulberry leaves using Response Surface Methodology (RSM). *Romanian Biotechnology of Letters*, 2012, 17, 7295–7308.

[8] Hraš, A.R., Hadolin, M., Knez, Z. and Bauman, D. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate

and citric acid in sunflower oil. *Food Chemistry*, 2000, 71: 229–233.
[9] Kadiri, O. and Olawoye, B. Underutilized indigenous vegetable (UIV) in Nigeria: a rich source of nutrient and antioxidants- a review. *Annals. Food Science and Technology*, 2015, 16(2): 307-318
[10] Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J,P., Pihlaja K, and Kujala, T.S. Antioxidant activity of plant extracts containing phenolic compounds, Journal of Agricultural and Food Chemistry, 1999, 47(10), 3954-3962.
[11] Liyana-Pathirana, C. M.; Shahidi, F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem. 2005, 93, 47–56
[12] Moskovitz, J., Yim, K.A. and Choke, P.B. Free radicals and disease. *Archives of Biochemistry and Biophysics* 2002, 397: 354–359
[13] Peng, X.Y., Xiong, Y.L. and Kong, B.H. Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. *Food Chemistry* 2009, 113: 196–201.
[14] Pownall, T. L., Udenigwe, C. C. and Aluko, R. E. Amino acid composition and antioxidant properties of pea seed (*Pisum sativum*L.) enzymatic protein hydrolysate fractions. *Journal of Agricultural Food Chemistry*, 2010, 58: 4712–4718.
[15] Prieto P, Pineda M, Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Bio* 1999, 269:337–341.
[16] Rao, G. Optimization of ultrasound-assisted extraction of cyanidin 3-rutinoside from litchi (*Lichi chinensis* Sonn.) fruit pericarp. *Analytical Methods* 2010, 2:1166–1170.
[17] Rodrigues, S., Pinto, G. A. S. and Fernandes, F. A. N. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell. Powder by response surface methodology. *Ultrasonics Sonochemistry*, 2008, 15: 95–100
[18] Saunders R, Becker R. Amaranth: a potential food and feed resource. Adv. Cereal Sci. Technology, 1984, 6: 57-96. Stadtman, E.R. Protein oxidation and aging. *Free Radical Research*, 2006, 40: 1250–1258.
[19] Triveni, R., Shamala, T. R. and Rastogi, N. K. Optimised production and utilisation of exopolysaccharide from *Agrobacterium radiobacter*. *Process Biochemistry*, 2001, 36: 787–795.
[20] Tsapatsaris, S. and Kotzekidou, P. Application of a central composite design and response surface methodology to the fermentation of olive juice by *Lactobacillus plantarum* and *Debaryomyces hansenii*. *International Journal of Food Microbiology*, 2004, 95: 157–168.
[21] Wang. S.Y. and Jiao. H. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry, Journal of Agricultural and Food Chemistry, 2000, 48(11), 5672-5676.
[22] Yang, B., Zhao, M. M., Shi, J., Yang, N. and Jiang, Y. M. Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chemistry*, 2008, 106: 685–690.
[23] Yim, H. S., Chye, F. Y., Koo, S. M., Matanjun, P., How, S. E. and Ho, C. W. Optimization of extraction time and temperature for antioxidant activity of edible wild mushroom, Pleurotus porrigens. *Food and Bioproducts Processing*, 2012, 90: 235–242
[24] Zhidong, L., Benheng, G., Xuezhong, C., Zhenmin, L., Yun, D., Hongliang, H and Wen, R. Optimisation of hydrolysis conditions for antioxidant hydrolysate production from whey protein isolates using response surface methodology. *Irish Journal of Agricultural and Food Research*, 2013, 52: 53–65.