

# Effect of Roasting on the Phytochemical Properties of Three Varieties of Marble Vine (*Dioclea reflexa*) Using Response Surface Methodology

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**ABSTRACT:** This study assessed the optimum roasting conditions on the phytochemical properties of three varieties of *Dioclea reflexa* seeds using response surface methodology. Roasting conditions were varied using temperature (110°C~200°C) and time (10~40 min). Phytochemical components (phenolics, tannin, flavonoids, cardiac glycoside, and steroids) of the seeds were screened and estimated. The study showed that availability of phytochemical activities was heat-dependent. An increase in roasting temperature beyond 110°C for 10 min resulted in a decrease in total phenolic (TP) and flavonoid (TF) contents. However, prolonged durations of roasting favored increased amounts of TP and TF in dark and light varieties. Total sterol, tannin, and cardiac glycoside contents increased with increasing roasting temperature and time. The desirability of the models were 0.76, 0.74, and 0.72 for black, dark brown, and light brown, respectively. The coefficients of regression ( $R^2$ ), ranged from 0.66 to 0.98 signifying accuracy of the model. The following models (cubic, quadratic, and 2 factor interaction) were significant ( $P \leq 0.05$ ). We found that roasting time influenced the phytochemical properties of *D. reflexa* to a greater extent than temperature. The optimum roasting temperature and time was found to be 110°C, 35 min, 40 min, and 32 min in black, dark brown, and light brown varieties, respectively. Roasting conditions significantly affects the phytochemical contents of three varieties of *D. reflexa* seed flour ( $P < 0.05$ ). Therefore, *D. reflexa* holds the potential to be used in development of functional foods and in therapeutic applications to promote health.

**Keywords:** phytochemicals, *Dioclea reflexa*, temperature, time, desirability

## INTRODUCTION

Phytochemicals in plants are considered potential agents that prevent or manage a wide range of diseases, such as neurodegenerative diseases, diabetes, cancer, and cardiovascular disorders (Soobrattee et al., 2005). These phytochemicals are widely distributed in the plant kingdom; since they are found in vegetables, fruits, and beverages, they are an integral part of the human diet (Luximon-Ramma et al., 2005). Occurrences of degenerative diseases such as cancer, hypertension, and diabetes are increasing in both developed and developing countries (Graham et al., 2006). Despite efforts made to reduce or manage the prevalence of diabetes it still remains a major healthcare challenge. Therefore, great attention has

been focused on the use of medicinal plants as alternative therapeutics for the management of these diseases such as diabetes. Ohnishi et al., (2004) reported that phenolics such as ferulic acid reduced blood glucose levels. Further, phytochemicals such as quercetin and its derivatives; rutin inhibits key enzymes linked to type 2 diabetes *in vitro* (Oboh et al., 2015). *Dioclea reflexa* is an underutilised legume that belongs to the family Leguminosae and sub-family Papilionoideae (Akinyede et al., 2017). *D. reflexa* is also known as sea beans, marble vine, horse eye, and Agba-arin by the Yorubas in South-Western of Nigeria, and Ukpo and Ebba by the Igbo's in the South-Eastern of Nigeria. The three known varieties of *D. reflexa* are dark brown, light brown, and black (Alabi and Alector, 2011). Due to the high saponification and

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iodine levels found in *D. reflexa* oil, it has gained applications in alkyl resin, shoe polish, liquid soap, and shampoo production (Jide, 2010). Therefore, *D. reflexa* oil could be good substitutes for the ever-increasing demands for conventional oils that are used for domestic and industrial purposes (Iliemene and Atawodi, 2014). The presence of some anti-nutritional components, such as trypsin inhibitors, phytic acid, tannins, saponin, and hemagglutinins, can cause adverse physiological responses or diminish the availability of nutrients, thereby interfering with digestion of carbohydrates and proteins (Oboh et al., 2010). However, such anti-nutritional properties can be reduced by dehulling, soaking, boiling, germination, and roasting operations (Keyata et al., 2018). Roasting process involves the application of thermal energy, which causes water to evaporate from biological materials. Roasting helps create the desired flavour, colour, texture, and acceptability of roasted products (Khan and Saini, 2016). However, damage as result of heat could be reduced due to the antioxidant nature of phytochemicals, which play an important role in protecting nuts and oilseeds against fat deterioration (Khan and Saini, 2016). Sharma et al. (2015) reported an increase in TP contents and antioxidant activities of six varieties of onion at 80°C, 100°C, and 120°C.

Response surface methodology (RSM) is an effective statistical tool used for determining the optimal conditions for complex processes (Kim, 2016). Roasting has been reported to aid the release of antioxidants from legumes; however, information about the optimal roasting condition of the three varieties of *D. reflexa* is lacking despite its potential. This study sought to determine the optimal roasting conditions to maintain maximal phytochemical and antioxidant activities in the three varieties of *D. reflexa* seeds.

## MATERIALS AND METHODS

### Materials

Marble vine (*D. reflexa*) seeds were sourced from an indigenous farmer in Akure, Ondo State, Nigeria. All the chemicals and reagents used were of analytical grade.

### Experimental design

Seeds were roasted using developed experimental runs from the central composite design of the RSM (Design-Expert version 8.3.0.1, Stat-Ease, Minneapolis, MN, USA). The upper and lower boundaries of roasting conditions used were 200°C for 40 min and 110°C for 10 min, respectively. The effects of the two independent variables [(roasting temperature (A) and roasting time (B)] on five responses [steroid content estimation, total phenolics (TP), total tannins (TT), total cardiac glycoside,

and total flavonoid (TF)] were evaluated as shown in Table 1. The models with statistically significant parameters ( $P \leq 0.05$ ) were considered, optimized using numerical tools and the desirability.

$$Y = \beta_0 + \sum_{i=1}^2 b_i X_i + \sum_{i=1}^2 b_i X_i^2 + \sum_{i=1}^2 \sum_{j=1}^2 b_{ij} X_i X_j \quad (1)$$

The second order polynomial equation (Eq. 1) was used to determine the effect of independent variables (process variables) on the responses.

### Sample preparations

Three varieties of *D. reflexa* seeds (200 g) were sorted, washed, and dried at ambient temperature for 2 days. Roasting was carried out in a hot air oven following the runs generated from design expert software, as shown in Table 1. Samples were cooled and pulverised using a hammer mill (model MS-223, Fritsch, Taipei, Taiwan). Samples were then sieved through a 250 µm screen and the resultant flour was stored in a plastic container. About 100 g of the sample was extracted through refluxing in 1.0 L of 99% ethanol for 48 h at room temperature. The sample was then filtered with a sieve of a considerable pore size. The clear solution obtained was then concentrated using a rotary evaporator (Rotavapor R-210 /215, BÜCHI Labortechnik AG, Flawil, Switzerland) and lyophilized with a freeze dryer (7752020, Labconco, Kansas City, MO, USA) to obtain a dried powdery concentrate.

### Qualitative phytochemical screening of *D. reflexa* seeds

The sample extract and its fractions were screened to qualitatively determine the amounts of phytochemicals using standard methods of analysis. The amounts of alkaloids, flavonoids, steroids, saponins, tannins, terpenoids, cardiac glycosides, and anthraquinones were determined

**Table 1.** Generated runs from central composite design for optimization of roasting in *Dioclea reflexa* varieties

Runs	Roasting temperature (°C)	Roasting time (min)
1	110	25
2	110	10
3	110	40
4	155	25
5	155	40
6	200	10
7	200	40
8	200	25
9	155	25
10	155	25
11	155	25
12	155	25
13	155	10

according to the method of Harborne, 1998; Yadav and Agarwala, 2011; Sofowora, 1982; Trease and Evans, 1989. TPs were determined using the Folin-Ciocalteu method (Singleton et al., 1999).

### Quantitative phytochemical screening of three varieties of *D. reflexa* seeds

**Determination of tannin content:** Tannin contents were determined by the Folin-Ciocalteu method, as described by Marinova and Ribarova (2005). Approximately 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, and 1 mL of 35% Na<sub>2</sub>CO<sub>3</sub> solution was made up to 10 mL mark with distilled water. The mixture was then mixed and maintained at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80, and 100 µg/mL) were prepared and the absorbance was measured against the blank at 725 nm using an ultraviolet (UV)-visible spectrophotometer. The tannin content was expressed in mg of gallic acid equivalents (GAE) of extract.

**Determination of alkaloid content:** The alkaloid concentration was determined by the method of Shamsa et al. (2008). Sample extract (1 mg) was dissolved in dimethyl sulphoxide, then 1 mL of 2 N HCl was added and the solution was filtered. The solution was then transferred into a separating funnel, where 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. The mixture was homogenized with 1, 2, 3, or 4 mL chloroform by vigorous shaking. The absorbances of the samples and standards were measured at 470 nm using an UV-visible spectrophotometer. The total alkaloid content was expressed as mg of alkaloid extract per gram (AE/g) of extract. TF content was measured by aluminium chloride colourimetric assays (Kaviarasan et al., 2007) and expressed in quercetin equivalents (QE). Cardiac glycosides were quantitatively determined in securidase equivalent per gram (SE/g), following the method of Solich et al. (1992) with some modifications. Steroid content was determined by the spectrophotometric method of Madhu et al. (2016) and expressed in catechin equivalents (CE).

### Statistical analysis

Data were analysed using RSM of the Design-Expert version 8.3.0.1 (Stat-Ease). The analysis of variance (ANOVA) of the regression coefficients of the fitted polynomial equations for each response variable was determined using the Statistical Package for Social Scientists (SPSS; version 17.0, SPSS Inc., Chicago, IL, USA). The *P*-value was used to evaluate the significance of model and terms. Three-dimensional (3D) response surface plots were drawn to illustrate the effects of the independent variables on the responses.

## RESULTS AND DISCUSSION

### Phytochemical screening

Phytochemicals in plants can be modified to more active forms upon exposure to considerable heat. Heating in the natural state can liberate phytochemicals from inert to active states with either therapeutic or toxic abilities. Prolonged exposure to high heat can result in degradation of phytochemicals into a less therapeutic state. Antioxidant activity may decrease at prolonged exposure to high temperatures and form pro-oxidants, which can alter some of the structure of the existing antioxidants. According to Sharma et al. (2015) TP contents and antioxidant activities of varieties of onion increase when roasted with increased temperature (80 to 150°C) for 30 min, suggesting that heating enhances antioxidant activity in certain fruits and vegetables. However, other findings show that apart from *Allium* species, the antioxidant activity of most food reduces with heating from 65°C to 100°C (Yin and Cheng, 1998). Phytochemical screening of the varieties of *D. reflexa* (black, light brown, and dark brown is presented in Table 2. At increased A and B, the following phytochemicals (alkaloids, terpenoids, anthraquinones, saponins, steroids, phenols, tannins, cardiac glycosides, and flavonoids) were detected. Similar observations reported by Ogundare and Olorunfemi (2007) indicated that phytochemical analysis of *D. reflexa*, *Mucuna pruriens*, *Ficus spherifolia*, and *Tragia spathulata* leaf extract revealed the presence of alkaloids, tannins, phenols, and glycosides.

Upon prolonged heating (200°C at 40 min and 200°C at 25 min), tannin was not detected in the light brown variety of *D. reflexa*. However, there was an increase in alkaloid, saponin, and steroid content as temperature increased with increased duration of roasting in the order of black, light brown, and dark brown varieties; for terpenoid content, the reverse trend was observed. Phytochemicals were more prominent in the black variety compared with the light and dark brown varieties. Thus, the potency of the seed varieties maybe predicted from the chemical compounds responsible for the desired therapeutic properties and consequential physiological effects in animals and humans.

### Quantification of phytochemicals in *D. reflexa* seeds

The effect of roasting conditions on the phytochemical contents of three varieties of *D. reflexa* seeds are presented in Table 3. For the black varieties, the steroid content estimation (SCE) values ranged from 352.27 to 454.60 µg/CE mL while the dark brown and light brown varieties ranged from 290.63 to 587.60 µg/CE mL and 299.81 to 550.93 µg/CE mL, respectively. Both A and B influenced the steroid content of *D. reflexa* seeds. For the dark brown variety, the A and B favoured the SCE con-

**Table 2.** Phytochemical screenings of *Dioclea reflexa* black (B), light brown (L), and dark brown (D) varieties, as affected by roasting temperature and time

Temperature/time	Alkaloid			Terpenoid			Anthraquinone			Saponin			Steroid			Phenolics			Tannin			Cardiac glycoside			Flavonoid		
	B	D	L	B	D	L	B	D	L	B	D	L	B	D	L	B	D	L	B	D	L	B	D	L	B	D	L
Raw	+++	+	+	+	+	+	+	+	+	++	++	++	+++	++	++	+++	++	++	++	++	+	+	+	+	++	+	++
110/25	+	+	+	+	++	—	+	+	+	+	++	+	++	++	++	+++	++	++	+	+	++	+	+	+	+	+	+
110/10	+++	+	+++	+	++	+	+	+	+	++	++	+++	++	++	+++	+++	++	+	+	+	+	++	+	++	+	+	+
110/40	++	+	+++	+	+	++	+	+	+	++	++	+++	+++	+++	+++	++	++	++	+	+	+	+	+	++	+	++	++
155/25	++	+	++	+	+	+	+	++	+	+	++	++	++	++	++	++	++	+	+	+	+	+	+	++	+	++	++
150/40	+	++	+	—	+	++	+	++	++	+	++	+	++	++	+++	++	++	+	+	+	+	+	+	++	+	++	++
200/10	+	+	+	—	+	++	—	++	+	+	++	++	++	++	+++	++	++	++	+	+	+	+	+	—	+	+	++
200/40	+	++	+++	++	+	++	++	++	++	+	++	++	++	++	++	++	++	++	+	+	—	+	—	+	+	+	++
200/25	+	++	+	++	+	++	++	+	++	++	++	++	+	++	++	++	++	++	+	+	—	+	+	—	+	+	+
150/10	++	+	+	+	+	+	+	+	+	++	++	++	++	++	+++	++	++	++	+	+	+	+	++	+	++	+	++

—, none; +, abundant; ++, more abundant; +++, most abundant.

tent, and was highest at 110°C for 40 min; whereas the optimal conditions for light brown variety was 155°C for 40 min (550.93 µg/CE mL). The lowest SCE was recorded for the light brown variety (299.81; µg/CE mL), which compared favourably with unroasted seeds. The SCE for black variety was lowest when roasted at 200°C for 10 min (352.27 µg/CE mL). Results obtained from the optimization process showed that the light brown variety is laden with steroids. Heat treatment is known to cause alterations to the chemical structures of certain molecules, including proteins that are associated with phenolic compounds (Wani et al., 2017).

The TP contents of three varieties of *D. reflexa* ranged from 310.50 to 317.00 mg GAE/g. For the black variety, the TP content was at its lowest at 200°C (310.50 mg GAE/g) and was at its highest 110°C for 25 min. The TP for the dark brown variety ranged from 315.33 to 317.0 mg GAE/g. For the light brown variety, the TP content ranged from 311.50 to 315.17 mg GAE/g, and was highest after roasting for 110°C for 25 min. However, there was no significant difference ( $P > 0.05$ ) in the TP content of the black, dark brown, and light brown varieties except with the control. It is known that high temperatures and low molecular weight phenolic compounds easily volatilise (Djikeng et al., 2018). These observations are consistent with the report of Rizki et al. (2015) within which the TP content of sesame seeds was shown to significantly ( $P \leq 0.05$ ) decrease with B.

The total tannin (TT) content of the three varieties of *D. reflexa* seeds ranged from 0.00 to 34.91 mg GAE/g. The TT content was highest after roasting at 110°C for 40 min for the black, dark brown, and light brown varieties (31.81, 34.91, and 32.70 mg GAE/g, respectively). At B longer than 25 min, and temperature above 155°C, TT content was negatively affected in all the three varieties. A similar observation was reported by Olapadea and Ajayia (2006) using roasted *Senna occidentalis* seeds. The TT

content reduced to zero at A of 200°C and B between 25 and 40 min for the light varieties.

The total cardiac glycoside (TCG) contents of the three varieties of *D. reflexa* seeds ranged from 0 to 0.63 mg SE/g, and was highest in the black variety compared with the other varieties. The TCG content increased with increasing process variables (A and B), except in the light brown variety and the unroasted seeds. For the light brown variety, the TCG content was highest after roasting at 155°C for 25 min (0.28 mg SE/g), whereas the TCG was highest for both the black and dark brown varieties after roasting at 200°C for 40 min.

The TF contents of the three varieties of *D. reflexa* are presented in Table 3. The TF content was lowest (17.33 mg QE/g) for the dark brown variety after roasting at 200°C for 25 min and was highest (49.78 mg QE/g) after roasting at 110°C for 10 min. For the light brown variety, the TF content was highest (46.78 mg QE/g) after roasting at 110°C for 25 min. The A and B influence the phytochemical contents of black, dark brown, and light brown varieties of *D. reflexa* seeds. Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants, which have been reported to exert potent antioxidant activity against superoxide radicals.

The results of optimisation of the effect of the responses (A and B) on the variables (estimation of the content of steroids, TP, TT, total cardiac glycosides, and TF) are presented in Table 4. For SCE, the models followed a cubic model in the black and dark brown varieties and quadratic in the light brown variety. A non-significant lack-of-fit model was applied, whereby the dark brown and light brown varieties had non-significant lack-of-fit. For the three varieties, A and  $AB^2$  were significant ( $P \leq 0.05$ ) model terms, whereas the light brown variety had an additional significant model term ( $A^2$ ). Since 'A' represents the temperature, the outcome of the model terms indicates that temperature had a greater effect on the SCE

**Table 3.** Effect of roasting conditions on the phytochemical contents of the three varieties of *Dioscorea reflexa* sseed flour

Run	Independent variables			Dependent variables													
	Temperature (°C)	Time (min)	SCE (µg/CE/mL)	Black variety				Dark variety				Light variety					
				TP (mg GAE/g)	TT (mg GAE/g)	TCG (mg SE/g)	TF (mg QE/g)	SCE (µg/CE mL)	TP (mg GAE/g)	TT (mg GAE/g)	TCG (mg SE/g)	TF (mg QE/g)	SCE (µg/CE mL)	TP (mg GAE/g)	TT (mg GAE/g)	TCG (mg SE/g)	TF (mg QE/g)
1	110	10	358.06	316.17	22.41	0.04	49.78	290.63	316.33	24.91	0.41	42.89	299.81	314.17	24.81	0.16	46.22
2	110	40	446.60	315.17	31.81	0.26	30.44	587.60	316.33	34.91	0.22	28.33	524.27	313.17	32.70	0.19	37.88
3	110	25	358.18	316.33	24.29	0.16	32.77	345.19	317.00	24.19	0.16	26.67	321.93	315.17	24.80	0.16	46.78
4	155	25	450.60	314.00	30.70	0.30	32.53	480.93	315.67	32.84	0.32	26.17	527.27	312.83	32.70	0.27	37.44
5	155	25	436.55	314.12	30.26	0.21	32.21	481.91	315.67	30.90	0.32	21.32	527.27	313.83	31.70	0.28	38.90
6	200	25	454.60	310.50	24.16	0.44	20.98	329.93	315.33	29.18	0.50	17.33	487.27	311.50	0.00	0.00	28.09
7	155	25	451.34	315.03	27.62	0.31	31.31	370.93	315.65	32.91	0.30	27.41	527.27	312.83	32.70	0.27	37.44
8	155	10	356.27	312.43	21.73	0.10	32.25	464.34	315.54	32.41	0.31	24.61	479.42	312.00	25.42	0.18	40.33
9	200	10	352.27	310.50	28.17	0.22	26.91	375.27	315.50	30.07	0.19	20.67	545.93	312.17	30.49	0.00	34.87
10	155	25	448.63	315.30	30.25	0.21	32.42	461.92	315.57	31.48	0.32	21.33	507.25	312.76	32.68	0.22	35.50
11	155	25	450.33	314.43	31.56	0.32	31.41	499.91	315.33	32.91	0.31	25.32	484.27	312.83	31.74	0.23	36.80
12	200	40	453.27	310.50	27.19	0.63	25.19	371.60	315.33	30.04	0.55	19.63	417.60	311.50	0.00	0.00	31.11
13	155	40	421.27	312.33	30.65	0.36	32.44	433.27	315.67	31.91	0.49	25.19	550.93	312.17	30.39	0.21	34.73
Con	—	—	450.27	263.33	24.95	0.43	20.67	356.93	180.12	25.47	0.56	16.67	440.60	196.42	24.91	0.21	25.88

Values are means of triplicate determination.

$P \leq 0.05$ .

Con, control; SCE, steroid content estimation; TP, total phenolics; TT, total tannins; TCG, total cardiac glycoside; TF, total flavonoid; CE, catechin equivalent; GAE, gallic acid equivalent; QE, quercetin equivalent; SE, securidase equivalent.

**Table 4.** Results of ANOVA analysis of the phytochemical content of the black (B), light brown (L), and dark brown (D) varieties of *Dioclea reflexa* seed flour

Parameters	Sample	Model	Lack-of-fit	R <sup>2</sup>	Adjusted R <sup>2</sup>	Equations	Significant model terms
SCE	B	Cubic	Significant	0.91	0.77	$20.97+1.20A+0.82B+0.08AB-0.37A^2-0.82B^2+0.36A^2B-1.20AB^2$	A, AB <sup>2</sup>
	D	Cubic	NS	0.81	0.67	$6.12-0.04A-0.04B-0.18AB-0.20A+0.21A^2B$	AB, A <sup>2</sup>
	L	Quadratic	NS	0.90	0.82	$515.45+50.80A+27.94B-88.20AB-93.82A^2+16.75B^2$	A, AB, A <sup>2</sup>
TP	B	Quadratic	NS	0.92	0.88	$314.32-2.70A-0.18B+0.25AB-0.26A^2-1.30B^2$	A, B <sup>2</sup>
	D	Quadratic	NS	0.87	0.79	$315.62-0.58A-6.67B-0.04AB+0.43A^2-0.13B^2$	A, A <sup>2</sup>
	L	Quadratic	NS	0.68	0.58	$17.70-0.03A-7.06B-0.2B^2$	A
TT	B	Quadratic	NS	0.66	0.50	$28.97+0.17A+2.89B-2.60AB-2.63A^2$	B
	D	Quadratic	Significant	0.75	0.56	$31.75+0.88A+1.58B-2.51AB-3.01A^2+1.57B^2$	AB, A <sup>2</sup>
	L	Quadratic	Significant	0.83	0.72	$30.03-8.64A-2.94B-9.60AB-12.94A^2+2.57B^2$	A, AB, A <sup>2</sup>
TCG	B	Quadratic	NS	0.93	0.91	$0.27+0.14A+0.15B+0.05AB$	A, B
	D	2FI	Significant	0.72	0.64	$0.57+0.06A+0.05B+0.12AB$	A, AB
	L	2FI	NS	0.95	0.91	$0.24-0.08A+1.00B-7.50AB-0.14A^2-0.03B^2$	A, A <sup>2</sup>
TF	B	Quadratic	Significant	0.88	0.80	$0.18+0.02A+7.57B-8.26AB+0.01A^2-9.10B^2$	A, B, A <sup>2</sup>
	D	Quadratic	NS	0.82	0.70	$3.16-0.25A-0.07B+0.09AB-0.03A^2+0.12B^2$	A
	L	Quadratic	NS	0.98	0.96	$37.33-9.35A-2.95B+1.14AB+0.19B^2+4.82AB^2$	A, B, AB, AB <sup>2</sup>

Significant  $P \leq 0.05$ .

SCE, steroid content estimation; TP, total phenolics; TT, total tannins; TCG, total cardiac glycoside; TF, total flavonoid; 2FI, two factor interaction; NS, not significant; A, roasting temperature; B, roasting time; R<sup>2</sup>, coefficient of regression.

than B. The results in Table 3 also strengthened the fact that higher roasting temperatures have more effect on SCE than B, as higher temperatures rather than longer times produced high SCE contents. The final equations (Table 4) also indicated that the coefficient of temperature had more positive values than those of B. The determination coefficient ( $R^2$ ) and adjusted  $R^2$  values were sufficient for a good model, and less deviation from the graphical fit described by Awolu et al. (2017) was observed. As required, the lack-of-fit models for all the varieties for TP were non-significant. The models for all the varieties were also quadratic. The significant ( $P \leq 0.05$ ) model terms for the three varieties were "A", whereas the black variety had additional significant model terms ( $B^2$ ) at  $P \leq 0.05$ . These imply that A has a greater effect on TP content than B for the dark and light varieties, thus high temperatures should yield greater amounts of TP.

However, for the black variety, both A and B affected the TP content. The  $R^2$  values ranged from 0.68 to 0.92 whereas the adjusted  $R^2$  values ranged from 0.58 to 0.88. These values were sufficient for a good model, with less deviation from the graphical fit. For all the roasted varieties, the greater amounts of phenolic compounds compared with the unroasted seeds (control) maybe as a result of cellular structure degradation during heat treatment and consequently release of bound phenolic compounds (Dorta et al., 2012). A similar observation was reported by Şahin et al. (2009), who showed that increased A decrease the phenolic content of carob kibbles. The

3D plot showing the effect of the relationship between A and B on TP and steroid content estimations are presented in Fig. 1.

Tannins form complexes with protein, thus precipitate proteins in the gut, reducing their digestibility; these can cause astringent reactions in the mouth which makes food unpalatable. Tannins can also interfere with dietary iron absorption (World Health Organization, 1996). The TT contents of all the varieties followed a quadratic model. The black variety had a model terms "B" with a non-significant lack-of-fit; this indicates that only time had a large effect on the TT content. The coefficient of B had more positive values than A, as presented in (Table 4), showing that B had a greater effect than A on the TT content of the black variety. Dark brown and light brown varieties showed a linear interaction between A and B. The  $R^2$  and adjusted  $R^2$  for the black, dark brown, and light brown varieties were 0.66 and 0.50, 0.75 and 0.56, and 0.83 and 0.72, respectively. Among the three varieties, the light brown variety coefficient of regression was the closest to one and was in reasonable agreement with the adjusted  $R^2$  value.

The TCG of the three *D. reflexa* varieties are presented in Table 4. The black and dark brown varieties followed a 2 factor interaction model, where the light brown variety followed quadratic model. The black and light brown varieties had a non-significant ( $P \geq 0.05$ ) lack-of-fit, which is required to fit the model. For the black and dark brown varieties, A and B were significant ( $P \leq 0.05$ ) mod-

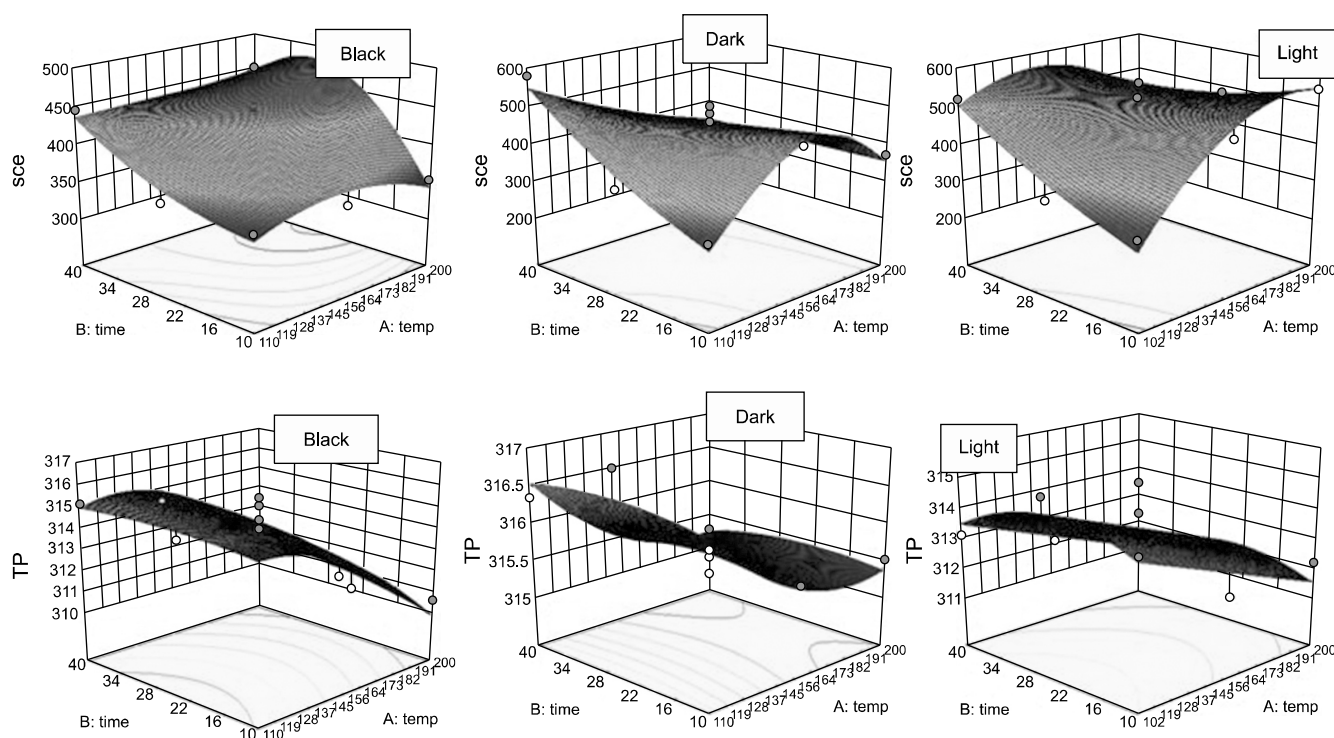


Fig. 1. 3D plot showing the effect of the relationship between the roasting temperature and time on steroid content estimations and total phenolic content.

el terms and had positive effects on total cardiac glycosides. Whereas for the light brown variety, only A affected TCG, as shown in the final equation (Table 4). The adequacy of the model fit is described by the closeness of both the  $R^2$  and adjusted  $R^2$  to one (Awolu et al., 2017). The  $R^2$  and adjusted  $R^2$  in the black and light brown varieties were 0.93 and 0.91, and 0.95 and 0.91, respectively, which both signify the model is adequate. The large regression coefficient of the model is indicative of a more significant effect on the response variables (Yang et al., 2009). The 3D plot showing the effect of the relationship between the A and B on tannin and total cardiac glycoside contents is presented in Fig. 2.

Flavonoid-rich foods have been reported to have a wide range of health-promoting effects, especially in the prevention and management of several diseases (Obloh et al., 2016). The models for the TF contents of the black and dark brown varieties of *D. reflexa* were quadratic, where-

as that for the light brown variety was cubic. The dark and the light brown varieties had a non-significant lack-of-fit with significant model terms (A) and (A, B, and  $A^2$ ), respectively. The TF content of the dark brown variety was only affected by A, with the  $R^2$  value of 0.82 and adjusted  $R^2$  value of 0.70 showing the adequacy of the model, whereas the light brown variety was greatly affected by A, B, the interaction between temperature and time (AB), and the quadratic interaction between temperature and time ( $AB^2$ ). The results in Table 4 show that all three varieties had a good  $R^2$  and adjusted  $R^2$ . The 3D plot shows the effect of the relationship between A and B on TF content, as presented in Fig. 3. Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants, and have potent antioxidant activity against superoxides. Our results showed that high temperatures reduced the TF contents of all the three seed varieties. Shamsa et al. (2015) showed that the TF con-

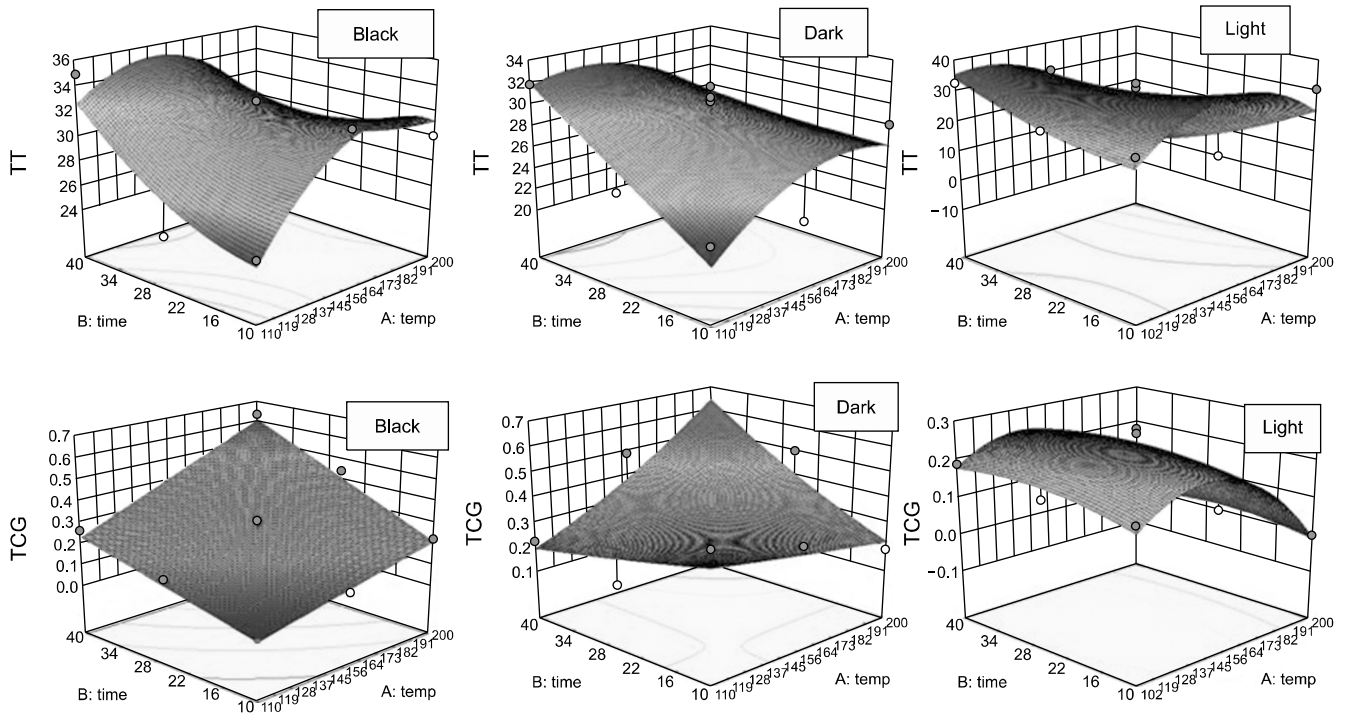


Fig. 2. 3D plot showing the effect of the relationship between roasting temperature and time on total tannin and total cardiac glycoside contents.

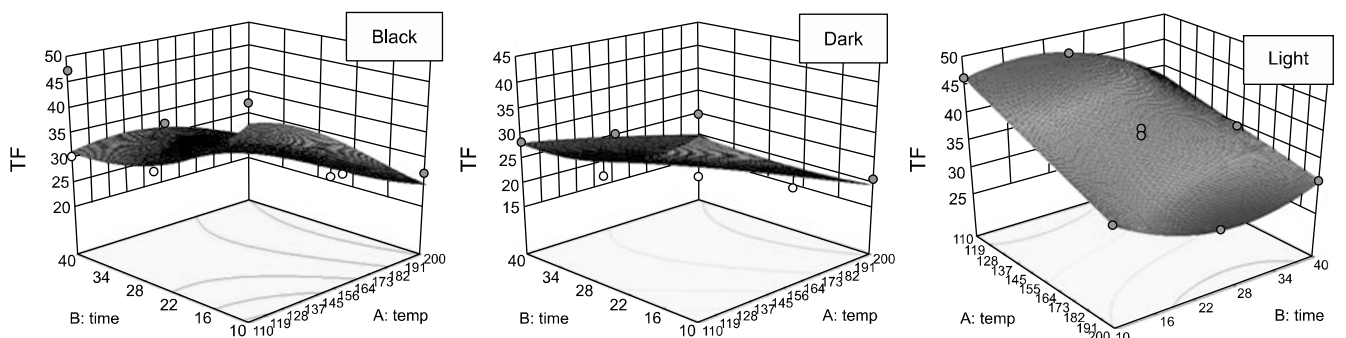


Fig. 3. 3D plot showing the effect of the relationship between roasting temperature and time on total flavonoid content.



tent do not show any regular trend, but that high-temperatures decrease flavonoid contents.

In conclusion, this study showed that phytochemicals are integral parts of plants that exhibit therapeutic properties that are used for management of several diseases including cancer, diabetes, cardiovascular disease, and erectile dysfunction. The phytochemical activities of three varieties of *D. reflexa* seed flour (light brown, dark brown, and black) with respect to A and B were investigated. The phytochemical contents of *D. reflexa* are influenced by seed variety and roasting conditions. We found that A above 110°C for duration of 10 to 40 min decreases the TP and TF contents of *D. reflexa* seeds flour. However, the TP and TF contents of the dark and light brown varieties were higher after prolonged durations of roasting. Increased A and durations increased total sterol, TT, and cardiac glycoside contents of all the three *D. reflexa* varieties. For the black and white varieties, the steroid content was highest after roasting at 200°C for 25 min, and at 155°C for 40 min, respectively. The B had a greater effect than A on the cardiac glycoside content of the seeds, however, for the light brown variety, increasing the A above 110°C resulted in destruction of the cardiac glycoside content. However, RSM analysis shows that roasting conditions significantly affect the phytochemical contents of all three varieties of *D. reflexa* seed flour ( $P < 0.005$ ). At a A of 110°C, the desirability values for the black, dark brown, and light brown varieties were 0.76, 0.74, and 0.72 for roasting durations of 35, 40, and 32 min, respectively. Therefore, optimised roasting conditions could be used to produce functional foods with potentials to promote better health.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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