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Effect of Autoclaving on Nutrients, Phenolics, Antioxidants, and Enzyme Inhibitory Properties of *Dioclea reflexa* Seed Flours

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Abstract: This study examined the impact of autoclaving on the nutritional, phytochemical, phenolic, antioxidant, and enzyme inhibitory properties of three varieties of *Dioclea reflexa* seed flours: light brown (LB), dark brown (DB), and black (B). Each variety was evaluated in raw form and after autoclaving for 10 and 30 min. Autoclaving led to a reduction in protein content, with the light brown variety maintaining the highest levels (24.94 to 21.44 g/100 g). Significant decreases in phytates (87.2%) and trypsin inhibitors (69.4%) were observed, especially in the light brown variety. Autoclaving significantly enhanced total phenolic content, particularly in the black variety, which showed the highest increases in catechin, quercetin, and chlorogenic acid. However, these increases were not accompanied by proportional improvements in antioxidant activity, indicating alterations in phenolic functionality. The light brown variety retained the highest antioxidant capacity based on the metal chelation assays. Enzyme inhibition studies revealed that autoclaving markedly improved α-amylase and α-glucosidase inhibitory activities, with the light brown variety showing the highest inhibition after 30 min (86.98% and 68.31%, respectively). These findings support the potential of autoclaved *D. reflexa* flours, especially the light brown and black varieties, as functional food ingredients for glycemic control.

Keywords: *Dioclea reflexa*; autoclaving; bioactive compounds; nutritional profile; underutilized legume

1. Introduction

Over the past few decades, there has been a growing emphasis on the potential of neglected and underutilized crops to contribute to global food security, human nutrition, and health [1]. Legumes have attracted considerable interest due to their high nutritional value, including high-quality proteins, essential amino acids, minerals, and an abundance of bioactive phytochemicals [2]. These bioactive compounds have been associated with various health-promoting effects such as improved gut health, enhanced glycemic control, and a reduced risk of chronic diseases [3]. Despite extensive research on common legumes like beans, lentils, and groundnuts, *Dioclea reflexa* (marble vine) remains underutilized and underexplored.

Dioclea reflexa is a perennial legume belonging to the family Fabaceae and subfamily Papilionoideae. It is locally known as sea bean, marble vine, horse eye, and *Agba-arin* among the Yoruba people of southwestern Nigeria, and as *Ukpo* and *Ebba* among the Igbo people of southeastern Nigeria, reflecting its long-standing traditional use in local food systems. The species is native to West and West-Central Tropical Africa, with documented occurrence in countries such as Nigeria, Ghana, and neighboring regions, and has also been reported in parts of South America [4,5]. The legume occurs in three distinct seed varieties: dark brown, light brown, and black. These seeds are traditionally used in local cuisines as soup thickeners and have been reported to possess functional properties that improve the texture and rheology of processed foods [4,6]. Despite its traditional use, *D. reflexa* is rarely cultivated or commercialized, and thus remains an underutilized legume. In addition, marble vine seeds contain a range of phytochemicals, including phenolic compounds, flavonoids, and known anti-nutritional factors such as tannins, protease inhibitors, and trypsin inhibitors [7]. However, to fully harness the nutritional and therapeutic potential of marble vine, it is essential to understand how processing affects its nutritional profile and bioactivity.

Thermal processing is one of the most widely applied approaches for improving the nutritional quality, safety, and functionality of legumes. Autoclaving is a cooking technique involving the application of high temperatures and pressures to food for a short period. This processing method is a convenient substitute for boiling and other laborious cooking methods that are often employed when preparing legumes for human consumption. It makes the products easy to use and has been shown to improve the safety, digestibility, and shelf life of legumes [8]. Several studies have investigated the impact of thermal processing on legumes and related foods. For instance, Nawaz et al. [9] found that autoclaving improved calcium availability and in vitro digestibility in fish bone powder, while Chisowa et al. [10] reported that autoclaving and boiling significantly reduced tannin content in soybean, pigeon pea, and cowpea. Ajatta et al. [11] demonstrated that roasting significantly increased the antioxidant activity of *D. reflexa* seeds when optimized using response surface methodology. Omotoso et al. [7] also found that soaking and boiling reduced anti-nutritional factors such as tannins and protease inhibitors in marble vine seeds. In addition, thermal processing has been reported to modify the phenolic composition of legumes, often by altering the extractability of bound phenolic compounds through changes in the food matrix [12,13]. Furthermore, processing duration and differences in color of the seed coat have been linked to variation in phenolic profiles, nutritional quality, and functional properties in legumes and pulses [1,11]. These observations suggest that the response of *D. reflexa* seed flours to autoclaving may depend on both processing conditions and seed variety.

Based on these reports, it was hypothesized that (i) autoclaving reduces the levels of anti-nutritional factors in *D. reflexa* seed flours due to the susceptibility of these compounds to heat and pressure; (ii) autoclaving alters the extractable phenolic profile and associated functional properties of *D. reflexa* seed flours; and (iii) autoclaving duration and seed variety influence the nutritional composition and bioactivity of *D. reflexa* seed flours. Therefore, this study aimed to evaluate the impact of autoclaving on the nutritional quality, phenolic composition, antioxidant activity, and carbohydrate-hydrolyzing enzyme inhibition potential of three varieties of marble vine seed flours. It is expected that this study will broaden the understanding of marble vine's value in developing sustainable, health-promoting food products and potentially in the dietary management of metabolic disorders such as diabetes.

2. Materials and Methods

2.1. Materials

Mature and healthy marble vine (*Dioclea reflexa*) seeds were purchased from a major open-air market in Akure, Nigeria. The seeds were authenticated at the Department of Crop, Soil, and Pest Management of the Federal University of Akure, Nigeria. Throughout the study, the chemicals used were of analytical grade and purchased from Sigma-Aldrich, London, United Kingdom.

2.2. Preparation of Flour Samples

A total of 600 g of seeds was used for each seed variety. The seeds were weighed and washed in a 1% sodium hypochlorite solution to remove surface contaminants, followed by rinsing with distilled water. Each 600 g seed batch per variety was divided into three equal portions (200 g each) to represent different treatment groups: raw, autoclaved for 10 min, and autoclaved for 30 min. Autoclaving was carried out in 1-L stainless steel containers containing 300 mL of distilled water (seed-to-water ratio 1:1.5, *w*/*v*), used to ensure complete immersion of the seeds during autoclaving and uniform heat transfer, at 121 °C and a gauge pressure of 15 psi (~103 kPa) using a laboratory autoclave. After autoclaving, seeds were cooled to room temperature, manually dehulled, and air-dried at ambient temperature (~25 °C) for 24 h. The dried seeds were milled using a Fritsch MS-223 hammer mill and sieved through a 250 µm mesh. The flour was stored in airtight plastic containers at 4 °C until analysis.

2.3. Chemical Analyses

2.3.1. Determination of Proximate Composition and Energy Value

Proximate composition was determined using AOAC [14] methods. Carbohydrate content was calculated by difference (100 − sum of measured proximate components), and energy value was estimated using Atwater conversion factors [15]

2.3.2. Determination of Minerals

Mineral elements were quantified following AOAC [14] procedures. Sodium and potassium were determined by flame emission photometry, phosphorus by the vanado-molybdate method, while calcium, iron, zinc, magnesium, manganese, and lead were analyzed using atomic absorption spectrophotometry.

2.3.3. Determination of Phytochemicals and Phytate/Minerals Molar Ratios

Tannin content was determined using the Folin–Denis method as described by Ejikeme et al. [16], and results were expressed as mg tannic acid equivalents per 100 g sample.

Trypsin inhibitor activity was evaluated according to Griffiths et al. [17] using BAPNA as substrate, and inhibitory activity was expressed as percentage reduction relative to the control.

Phytic acid content was determined using the method of Bedos et al. [18], while oxalate content was quantified following AOAC [14] procedures.

Total saponins were measured using the vanillin–sulfuric acid colorimetric method described by Makkar et al. [19], expressed as diosgenin equivalents.

Total flavonoid content was determined using the Aluminum chloride colorimetric method [20] and expressed as mg quercetin equivalents per gram of sample (mg QE/g).

2.3.4. Determination of Antioxidant Activity

DPPH, ABTS, Fe^{2+} chelation, and hydroxyl radical scavenging assays were carried out following standard procedures reported by Gyamfi et al. [21], Re et al. [22], Xie et al. [23], and Girgih et al. [24], respectively, with minor modifications. Antioxidant capacity was quantified spectrophotometrically and expressed as percentage inhibition.

2.3.5. Determination of Phenolic Profile

Briefly, 5 g of the powdered sample was extracted with 100 mL of distilled water (solid-to-solvent ratio 1:20, *w*/*v*) and shaken at room temperature for 4 h. The extract was centrifuged at 5000 rpm for 10 min to obtain a clear supernatant, which was then filtered through a 0.45 µm membrane filter prior to HPLC analysis. Phenolic compounds were analyzed using a Shimadzu Prominence HPLC system with an SPD-M20A diode array detector. Separation was achieved on a C18 column (4.6 × 150 mm, 5 µm) using a gradient of 1% formic acid in water (Solvent A) and methanol (Solvent B). The gradient ranged from 12% to 70% Solvent B over 80 min at a flow rate of 0.6 mL/min. Samples and mobile phases were filtered (0.45 µm) and degassed before injection (50 µL) at 15 mg/mL concentration. Detection wavelengths were 280 nm (catechin, epicatechin), 327 nm (caffeic acid), and 365 nm (flavonoids). Compounds were identified by retention time and UV spectra compared to standards and results were expressed as mg/kg. The method was adopted from Omoba et al. [25], where validation parameters including limits of detection (LOD) and quantification (LOQ) were reported.

2.3.6. Carbohydrate-Hydrolyzing Enzyme Inhibition Assays

Aqueous extracts of the flour samples were prepared and assessed for α-amylase and α-glucosidase inhibitory activities using the method of Kwon et al. [26] with slight modifications. The assays were carried out under standard incubation conditions, and enzyme inhibition was expressed as percentage reduction in activity relative to control reactions without extract.

2.4. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was used to evaluate multivariate relationships among proximate composition, mineral content, antinutritional factors, antioxidant activities, and enzyme inhibitory properties of *Dioclea reflexa* seed flours. All variables were standardized prior to analysis, and PCA was performed using the correlation matrix. Principal components with eigenvalues greater than 1.0 were retained. Factor loadings were used to identify variables contributing to each component, and a PCA biplot was generated to visualize sample distribution and variable associations.

2.5. Statistical Analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS version 21.0). Data were subjected to one-way analysis of variance, and mean differences were separated using Duncan's multiple range test at $p < 0.05$. Principal Component Analysis (PCA) was performed using Minitab version 21. Antioxidant correlation analyses and all graphical visualizations were carried out using GraphPad Prism version 8.

3. Results

3.1. Proximate Composition of Autoclaved Marble Vine Seed Flour

The proximate composition of raw and autoclaved flours from the three marble vine seed varieties is shown in Table 1. The crude protein content (g/100 g) of the flours varied significantly ($p < 0.05$), with the light brown variety having the highest (24.94 g) and the black variety having the least (12.56 g). The total protein in the samples ranged from 21.44 g in ALB30 to 24.94 g in RLB for the light brown seed, 13.56 g in ADB30 to 20.57 g in RDB for the dark brown seed, and 12.56 g in AB30 to 16.19 g in RB for the black seed. Autoclaving led to a significant decrease in total protein content of all samples with increasing autoclaving time. The level of moisture (g/100 g) also differs across the species and the minimum (4.52 g) and maximum (8.78 g) values were found in ALB30 and RDB, respectively. A slight decline in the moisture content was observed with prolonged autoclaving. A similar trend was seen in the fat content; the values decreased from 4.24 g to 2.17 g for LB, 4.97 g to 2.57 g for DB, and 3.86 g to 3.02 g for B, as the autoclaving time rises. For the total ash content (g/100 g) of the flours, values ranging between 2.73 g and 3.27 g for LB, 3.41 g and 3.61 g for DB, and 3.88 g and 3.99 g for B, were observed. The ash content exhibited a gradual increase as the autoclaving time was extended. The energy values showed slight variations between the three species and the autoclaving conditions. The energy values (kcal/100 g) ranged from 379.25 kcal in ALB30 to 383.52 kcal in RLB, 372.72 kcal in ADB10 to 373.85 kcal in ADB30, and 373.78 kcal in AB30 to 379.38 kcal in RB. The energy values of autoclaved samples were lower than those of control samples, with a notable decrease as the autoclaving time was prolonged.

Table 1. Proximate composition (g/100 g) and energy value (kcal/100 g) of autoclaved marble vine (*Dioclea reflexa*) seed flours and control samples.

Samples	Moisture	Total Ash	Crude Fat	Crude Fibre	Crude Protein	Carbohydrate	Energy
RLB	6.36 ± 0.77 ^{b}	2.73 ± 0.09 ^{d}	4.24 ± 0.03 ^{b}	0.33 ± 0.01 ^{d}	24.94 ± 0.44 ^{a}	61.40 ± 0.21 ^{d}	383.52 ± 2.81 ^{a}
ALB10	4.75 ± 0.43 ^{c}	2.79 ± 0.19 ^{d}	3.14 ± 0.19 ^{c}	0.18 ± 0.01 ^{e}	23.81 ± 0.44 ^{b}	64.70 ± 1.25 ^{c}	382.30 ± 1.51 ^{a}
ALB30	4.52 ± 1.21 ^{bc}	3.27 ± 0.12 ^{bc}	2.17 ± 1.07 ^{d}	0.11 ± 0.01 ^{f}	21.44 ± 0.43 ^{c}	68.49 ± 1.95 ^{b}	379.25 ± 0.03 ^{b}
RDB	8.78 ± 0.67 ^{a}	3.41 ± 0.08 ^{bc}	4.97 ± 0.23 ^{a}	0.70 ± 0.01 ^{b}	20.57 ± 0.43 ^{d}	61.57 ± 1.41 ^{d}	373.29 ± 1.81 ^{c}
ADB10	6.08 ± 0.56 ^{bc}	3.49 ± 0.07 ^{bc}	2.72 ± 0.53 ^{d}	0.09 ± 0.01 ^{f}	16.69 ± 0.44 ^{f}	70.37 ± 0.71 ^{ab}	372.72 ± 0.13 ^{c}
ADB30	6.07 ± 1.50 ^{bc}	3.61 ± 0.05 ^{cd}	2.57 ± 0.66 ^{d}	0.07 ± 0.01 ^{g}	13.56 ± 0.43 ^{f}	74.12 ± 1.67 ^{a}	373.85 ± 2.50 ^{bc}
RB	5.54 ± 1.46 ^{bc}	3.88 ± 0.19 ^{a}	3.86 ± 0.22 ^{b}	0.56 ± 0.01 ^{c}	16.19 ± 0.43 ^{e}	69.97 ± 2.29 ^{ab}	379.38 ± 5.44 ^{ab}
AB10	5.42 ± 0.18 ^{bc}	3.94 ± 0.36 ^{a}	3.25 ± 0.59 ^{bc}	0.87 ± 0.06 ^{a}	14.69 ± 0.44 ^{f}	71.83 ± 1.15 ^{a}	375.33 ± 2.47 ^{bc}
AB30	5.39 ± 0.51 ^{b}	3.99 ± 0.21 ^{a}	3.02 ± 1.26 ^{c}	0.95 ± 0.01 ^{a}	12.56 ± 0.43 ^{f}	74.09 ± 2.39 ^{a}	373.78 ± 3.50 ^{bc}

Values are Mean ± standard deviation. Mean values with the same superscript along the same column are not significantly different at $p < 0.05$. RLB: raw light brown; ALB10 and ALB30: light brown seeds autoclaved for 10 and 30 min; RDB: raw dark brown; ADB10 and ADB30: dark brown seeds autoclaved for 10 and 30 min; RB: raw black; AB10 and AB30: black seeds autoclaved for 10 and 30 min.

3.2. Mineral Composition of Autoclaved Marble Vine Seed Flour

Based on the results obtained for mineral composition (mg/100 g) of autoclaved and control samples of marble vine seed flours (Table 2), all species exhibited appreciable amounts of minerals. However, the mineral content was significantly reduced ($p < 0.05$) after autoclaving, except for Lead (Pb), which was not detected at all. In this study, the potassium content ranged from 125.40 mg in ALB30 to 202.50 mg in RLB, 181.50 mg in ADB30 to 339.00 mg in RDB, and 167.10 mg in AB30 to 235.80 mg in RB. The raw samples (RDB, RB, and RLB) generally had higher potassium content compared to the autoclaved ones (ALB30 and ADB30), regardless of their species. The sodium levels reduced from 89.10 mg in RLB to 2.70 mg in ALB30 for the light brown variety, 69.40

mg in RDB to 3.80 mg in ADB30 for the dark brown variety, and 76.20 mg in RB to 4.02 mg in AB30 for the black variety after autoclaving the raw samples for 30 min. The highest and lowest sodium content was observed in the light brown variety before and after autoclaving for 30 min respectively. Regarding calcium, the dark brown variety (3.36 mg–5.17 mg) had a higher calcium content than the light brown (1.38 mg–5.01 mg) and black (1.46 mg–1.95 mg) species before and after autoclaving. Nonetheless, autoclaving significantly reduced calcium content in all varieties, with samples autoclaved for 30 min dropping by up to 69% (ALB30). The phosphorus content of the autoclaved samples ranged from 14.01 mg in ALB30 to 15.17 mg in RLB, 15.36 mg in ADB30 to 16.87 mg in RDB, and 17.43 mg in AB30 to 20.39 mg in RB. RB exhibited the highest phosphorus content, while ALB30 had the lowest. The potassium content of all the samples reduced considerably after the autoclaving process. Similarly, the Ca/P ratio in the autoclaved samples ranged from 0.09 mg in ADB10 and ALB30 to 0.75 in RB.

Table 2. Mineral composition (mg/100 g) of autoclaved marble vine *(Dioclea reflexa)* seed flours and control samples.

Minerals	RLB	ALB10	ALB30	RDB	ADB10	ADB30	RB	AB10	AB30
Zn	1.92 ± 0.01 [b]	1.58 ± 0.01[c]	0.82 ± 0.02 [g]	1.52 ± 0.01 [d]	0.70 ± 0.01 [h]	0.70 ± 0.01 [h]	2.22 ± 0.01 [a]	1.45 ± 0.01 [e]	1.23 ± 0.04 [f]
K	202.50 ± 0.07 [c]	139.80 ± 0.07 [f]	125.40 ± 0.01 [g]	339.00 ± 0.14 [a]	202.20 ± 0.01 [c]	181.50 ± 0.07 [d]	235.80 ± 0.02 [b]	202.50 ± 0.09 [c]	167.10 ± 0.07 [e]
Ca	5.01 ± 0.02 [b]	4.01 ± 0.01 [c]	1.38 ± 0.03 [g]	5.17 ± 0.01 [a]	5.05 ± 0.02 [b]	3.36 ± 0.01 [d]	1.95 ± 0.03 [e]	1.95 ± 0.02 [e]	1.46 ± 0.01 [f]
Fe	0.95 ± 0.03 [e]	0.90 ± 0.01 [e]	0.84 ± 0.01 [g]	1.82 ± 0.02 [b]	1.37 ± 0.02 [d]	0.85 ± 0.01 [g]	1.92 ± 0.02 [a]	1.42 ± 0.02 [c]	0.86 ± 0.02 [f]
Mn	1.25 ± 0.03 [a]	0.23 ± 0.02 [d]	0.23 ± 0.02 [d]	0.27 ± 0.01 [c]	0.27 ± 0.02 [c]	0.23 ± 0.01 [d]	0.33 ± 0.02 [b]	0.30 ± 0.01 [b]	0.19 ± 0.01 [e]
Na	89.10 ± 0.03 [a]	2.84 ± 0.01 [h]	2.70 ± 0.07 [i]	69.40 ± 0.01 [c]	45.60 ± 0.14 [d]	3.80 ± 0.07 [g]	76.20 ± 0.07 [b]	7.87 ± 0.03 [e]	4.02 ± 0.02 [f]
Mg	4.15 ± 0.01 [b]	2.10 ± 0.07 [e]	1.90 ± 0.03 [f]	1.82 ± 0.02 [g]	1.77 ± 0.02 [h]	1.31 ± 0.01 [i]	8.90 ± 0.14 [a]	3.65 ± 0.07 [c]	3.22 ± 0.01 [d]
P	15.17 ± 0.02 [g]	14.18 ± 0.02 [i]	14.01 ± 0.01 [j]	16.87 ± 0.02 [d]	16.65 ± 0.02 [e]	15.36 ± 0.02 [f]	20.39 ± 0.02 [a]	19.83 ± 0.02 [b]	17.43 ± 0.02 [c]
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND
Na/K	0.44 [a]	0.02 [f]	0.02 [f]	0.21 [d]	0.23 [c]	0.02 [f]	0.32 [b]	0.04 [e]	0.02 [f]
Ca/P	0.33 [b]	0.28 [d]	0.09 [f]	0.31 [c]	0.30 [c]	0.22 [e]	0.75 [a]	0.09 [f]	0.08 [f]

Values are Mean ± standard deviation. Mean values with the same superscript along the same column are not significantly different at $p < 0.05$. ND: Not Detectable. RLB: raw light brown; ALB10 and ALB30: light brown seeds autoclaved for 10 and 30 min; RDB: raw dark brown; ADB10 and ADB30: dark brown seeds autoclaved for 10 and 30 min; RB: raw black; AB10 and AB30: black seeds autoclaved for 10 and 30 min.

3.3. Phytochemical Composition of Autoclaved Marble Vine Seed Flour

The flour samples obtained from three varieties of marble vine seeds contained various phytochemicals (mg/100 g), including saponin, phytate, flavonoid, tannin, oxalate, and trypsin inhibitors (Table 3). The concentrations of these phytochemicals ranged from 101 to 954 mg/100 g for tannin, 836 to 2391 mg/100 g for oxalate, 16 to 521 mg/100 g for phytate, 12 to 176 mg/100 g for saponin, 1200 to 3008 mg/100 g for flavonoid, and 734 to 2911 mg/100 g for trypsin inhibitors. In terms of tannin, it was observed that its content decreased with increasing autoclaving time for all varieties. For instance, the raw flour samples had higher tannin content compared to those autoclaved for 30 min. Among the three species, the light brown variety had the lowest tannin content both before (666 mg) and after (101 mg) autoclaving. The results also revealed a notable decrease in trypsin inhibitor in the flour samples after autoclaving, irrespective of the species. After autoclaving the flour samples for 30 min, trypsin inhibitors reduced by up to 69.4%, 37.68%, and 37.03% in light brown, dark brown, and black species, respectively. The highest level of trypsin inhibitor was found in the black species, followed by the dark brown and light brown species. The flavonoid in the samples also reduced after autoclaving but was more abundant in the light brown than the other two varieties. In addition, the levels of phytate in the samples dropped significantly from 480 mg to 61 mg (LB), 519 mg to 193 mg (DB), and 521 mg to 280 mg (B) after autoclaving the flour samples for 30 min. The inhibitory effect of phytate in the flour samples on bioavailability of minerals is predicted using the calculated phytate/minerals molar ratios shown in Table 3. The molar ratios of Iron (Phy/Fe: 6.16–43.12), Calcium (Phy/Ca: 2.78–16.18), and Zinc (Phy/Zn: 7.33–47.85) in all the marble vine flour samples, except ALB 10 and ALB 30, were higher than the critical values.

Table 3. Phytochemicals composition (mg/100 g) and phytate/mineral molar ratios of autoclaved marble vine *(Dioclea reflexa*) seed flours and control samples.

(A) Phytochemicals Composition (mg/100 g)										
Phytochemicals	**RLB**	**ALB10**	**ALB30**	**RDB**	**ADB10**	**ADB30**	**RB**	**AB10**	**AB30**	
Tannin	666 ± 0.18 ^{c}	327 ± 0.10 ^{e}	101 ± 0.05 ^{g}	878 ± 0.19 ^{b}	528 ± 0.12 ^{d}	289 ± 0.07 ^{f}	954 ± 0.10 ^{a}	681 ± 0.09 ^{c}	300 ± 0.04 ^{e}	-
Oxalate	1583 ± 0.05 ^{d}	1084 ± 0.19 ^{f}	836 ± 0.12 ^{g}	1980 ± 0.07 ^{b}	1367 ± 0.09 ^{e}	1089 ± 0.07 ^{f}	2391 ± 0.09 ^{a}	1725 ± 0.11 ^{c}	1569 ± 0.23 ^{d}	-
Saponin	103 ± 0.03 ^{d}	50 ± 0.03 ^{g}	12 ± 0.02 ^{h}	158 ± 0.02 ^{b}	102 ± 0.05 ^{d}	75 ± 0.07 ^{f}	176 ± 0.03 ^{a}	128 ± 0.06 ^{c}	86 ± 0.03 ^{e}	-
Flavonoid	3008 ± 0.24 ^{a}	2852 ± 0.19 ^{b}	2166 ± 0.07 ^{e}	2753 ± 0.17 ^{c}	2385 ± 0.12 ^{d}	1892 ± 0.07 ^{f}	2179 ± 0.08 ^{e}	1577 ± 0.14 ^{g}	1200 ± 0.08 ^{h}	-
Trypsin Inhibitors	2398 ± 0.04 ^{c}	1027 ± 0.09 ^{g}	734 ± 0.11 ^{h}	2853 ± 0.07 ^{b}	2280 ± 0.13 ^{d}	1778 ± 0.09 ^{f}	2911 ± 0.09 ^{a}	2288 ± 0.10 ^{d}	1833 ± 0.20 ^{e}	-
Phytate	481 ± 0.13 ^{b}	184 ± 0.09 ^{g}	61 ± 0.06 ^{h}	519 ± 0.03 ^{a}	338 ± 0.27 ^{d}	193 ± 0.05 ^{f}	521 ± 0.07 ^{a}	371 ± 0.16 ^{c}	280 ± 0.19 ^{e}	-
(B) Phytate/Mineral (Ca, Zn & Fe) Molar Ratios										
Ratios	**RLB**	**ALB10**	**ALB30**	**RDB**	**ADB10**	**ADB30**	**RB**	**AB10**	**AB30**	*** CV**
Phy/Ca	5.82 ± 0.01 ^{d}	2.78 ± 0.01 ^{g}	2.67 ± 0.01 ^{g}	6.08 ± 0.01 ^{c}	4.05 ± 0.01 ^{e}	3.48 ± 0.01 ^{f}	16.18 ± 0.01 ^{a}	11.51 ± 0.01 ^{b}	11.62 ± 0.01 ^{b}	0.24
Phy/Zn	24.70 ± 0.01 ^{e}	11.47 ± 0.01 ^{h}	7.33 ± 0.01 ^{i}	33.75 ± 0.01 ^{b}	47.85 ± 0.01 ^{a}	27.32 ± 0.03 ^{c}	23.08 ± 0.01 ^{f}	25.20 ± 0.01 ^{cd}	22.44 ± 0.01 ^{g}	15
Phy/Fe	43.12 ± 0.01 ^{a}	17.32 ± 0.01 ^{h}	6.16 ± 0.04 ^{i}	24.19 ± 0.01 ^{c}	20.98 ± 0.01 ^{f}	19.23 ± 0.01 ^{g}	23.01 ± 0.01 ^{d}	22.19 ± 0.01 ^{e}	27.55 ± 0.01 ^{b}	>1
Phy*Ca/Zn	3.09 ± 0.04 ^{c}	1.15 ± 0.01 ^{f}	0.25 ± 0.01 ^{h}	4.35 ± 0.01 ^{b}	6.00 ± 0.01 ^{a}	2.28 ± 0.03 ^{d}	1.13 ± 0.01 ^{f}	1.23 ± 0.01 ^{e}	0.82 ± 0.02 ^{g}	>200

Values are Mean ± standard deviation. Mean values with the same superscript along the same column are not significantly different at $p < 0.05$. RLB: raw light brown; ALB10 and ALB30: light brown seeds autoclaved for 10 and 30 min; RDB: raw dark brown; ADB10 and ADB30: dark brown seeds autoclaved for 10 and 30 min; RB*:* raw black; AB10 and AB30: black seeds autoclaved for 10 and 30 min. * CV: Critical values; Phytate:calcium = 0.24; Phytate:zinc = 15; Phytate:iron ≥ 1; phytate: calcium/zinc > 200 [27].

3.4. Phenolic Profile of Autoclaved Marble Vine Seed Flour

The phenolic composition of raw and autoclaved *Dioclea reflexa* seed flours is presented in Table 4. A total of fifteen phenolic compounds were identified and quantified across all seed varieties (light brown, dark brown, and black) in raw samples and in those autoclaved for 10 and 30 min. The identified compounds included flavonoids (catechin, epicatechin, quercetin, luteolin, kaempferol, naringenin, myricetin, and rutin) and phenolic acids (gallic acid, p-coumaric acid, p-hydroxybenzoic acid, caffeic acid, chlorogenic acid, syringic acid, and ferulic acid). Catechin was the most abundant phenolic compound across all samples, with concentrations ranging from 80.68 mg/kg in raw dark brown seeds (RDB) to 119.66 mg/kg in black seeds autoclaved for 10 min (AB10). Other prominent phenolics included gallic acid (29.05–51.33 mg/kg), chlorogenic acid (48.79–71.39 mg/kg), and quercetin (28.63–55.99 mg/kg). Autoclaving significantly increased the concentrations of most phenolic compounds, particularly in the light brown and black seed flours. For example, in black seeds, quercetin increased from 28.63 mg/kg in the raw sample (RB) to 55.99 mg/kg after autoclaving for 10 min (AB10), while chlorogenic acid increased from 48.79 mg/kg to 71.39 mg/kg under the same conditions. Among the three seed types, the black variety exhibited the most pronounced response to autoclaving. Total phenolic content increased following autoclaving in all seed varieties. In black seeds, total phenolics increased from 281.31 mg/kg in the raw sample to 466.15 mg/kg and 438.56 mg/kg after autoclaving for 10 and 30 min, respectively. Among all samples, the 10-min autoclaved black seed flour (AB10) recorded the highest total phenolic content (466.15 mg/kg), followed by the 10-min autoclaved light brown (ALB10: 414.15 mg/kg) and dark brown (ADB10: 381.35 mg/kg) samples. In addition, Figure 1 illustrates the relative contribution of individual phenolic compounds to total phenolics in raw and autoclaved samples, highlighting differences among seed varieties and autoclaving durations.

Table 4. Phenolic profile (mg/kg) of autoclaved marble vine *(Dioclea reflexa*) seed flours and control samples.

Phenolic Profile	RLB	ALB10	ALB30	RDB	ADB10	ADB30	RB	AB10	AB30
Catechin	94.11 g	103.56 d	105.89 c	80.68 i	97.38 e	95.49 f	90.68 h	119.66 a	107.55 b
p-coumaric acid	1.48 g	3.08 c	2.93 d	1.22 h	2.74 e	2.57 f	0.87 i	3.62 a	3.29 b
Epicatechin	5.31 g	7.11 c	6.86 d	5.03 h	6.15 e	6.02 f	4.81 i	7.80 a	7.53 b
p-hydroxybenzoic acid	6.72 g	8.77 c	8.38 d	6.18 h	8.19 e	8.00 f	6.01 i	9.11 a	9.02 b
Gallic acid	39.05 g	46.11 c	45.37 d	33.92 h	41.06 e	40.33 f	29.05 i	51.33 a	48.92 b
Caffeic acid	7.88 d	8.05 c	7.85 e	7.42 g	7.54 f	7.55 f	7.03 h	8.99 a	8.14 b
Syringic acid	4.81 g	5.39 c	5.25 d	4.60 h	5.12 e	5.09 f	4.27 i	5.93 a	5.53 b
Ferulic acid	3.09 g	4.88 c	4.57 d	2.92 h	4.28 e	4.11 f	2.66 i	5.11 a	5.03 b
Luteolin	22.91 g	30.14 c	29.47 d	19.15 h	27.84 e	26.18 f	18.03 i	38.63 a	35.83 b
Naringenin	1.53 g	3.02 c	2.86 d	1.32 h	2.74 e	2.74 f	1.11 i	3.39 a	3.12 b
Kaempferol	30.77 f	37.19 c	36.30 d	25.67 h	33.51 e	30.11 g	19.75 i	41.66 a	40.40 b
Quercetin	41.66 g	50.39 c	49.59 d	33.10 h	48.57 e	44.38 f	28.63 i	55.99 a	53.85 b
Chlorogenic acid	60.33 g	68.59 c	66.48 d	56.81 h	63.91 e	60.48 f	48.79 i	71.39 a	70.11 b
Myricetin	15.93 g	20.33 c	20.14 d	11.93 h	19.84 e	19.35 f	9.51 i	23.99 a	21.75 b
Rutin	12.45 f	17.54 c	15.86 d	10.93 h	12.48 e	11.79 g	10.11 i	19.55 a	18.49 b
Total	348.03 g	414.15 c	407.80 d	300.88 h	381.35 e	364.19 f	281.31 i	466.15 a	438.56 b

Values are Mean ± standard deviation. Mean values with the same superscript along the same column are not significantly different at $p < 0.05$. *RLB*: raw light brown; *ALB10* and *ALB30*: light brown seeds autoclaved for 10 and 30 min; *RDB*: raw dark brown; *ADB10* and *ADB30*: dark brown seeds autoclaved for 10 and 30 min; *RB*: raw black; *AB10* and *AB30*: black seeds autoclaved for 10 and 30 min.

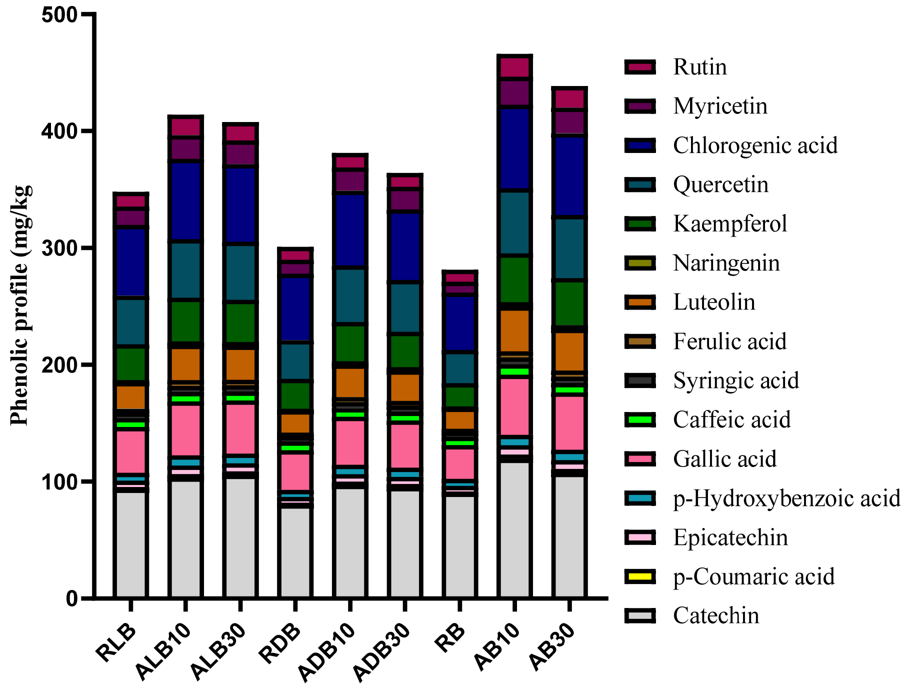

Figure 1. Relative distribution of individual phenolic compounds in raw and autoclaved *Dioclea reflexa* seed flours (light brown, dark brown, and black varieties). RLB*:* raw light brown; ALB10 and ALB30*:* light brown seeds autoclaved for 10 and 30 min; RDB*:* raw dark brown; ADB10 and ADB30: dark brown seeds autoclaved for 10 and 30 min; RB*:* raw black; AB10 and AB30*:* black seeds autoclaved for 10 and 30 min.

3.5. Antioxidant Activities of Autoclaved Marble Vine Seed Flour

The antioxidant capacity of raw and autoclaved *Dioclea reflexa* seed flours, as evaluated by four different assays; DPPH, ABTS, Fe^{2+} chelation and hydroxyl radical scavenging activity, is presented in Table 5. Generally, raw seed flours exhibited significantly higher ($p < 0.05$) antioxidant activity than those autoclaved across all seed varieties and assays. Among the raw samples, light brown seeds (RLB) demonstrated the highest antioxidant activity in all five assays: DPPH (79.37%), ABTS (58.71%), Fe^{2+} chelation (68.91%), and hydroxyl radical scavenging (61.38%). These were followed by raw black (RB) and raw dark brown (RDB) samples. Autoclaving led to a gradual decline in antioxidant capacity with increasing time. In the light brown variety, DPPH inhibition decreased from 79.37% (RLB) to 70.30% (ALB10) and 67.06% (ALB30). Similar patterns were observed in the ABTS assay (from 58.71% to 45.74%) and other antioxidant tests. In dark brown seeds, DPPH dropped from 74.82% (RDB) to 70.65% (ADB10) and further to 66.32% (ADB30). The most pronounced reductions were seen in the black variety, where DPPH decreased from 73.99% (RB) to 61.76% (AB30), and ABTS from 52.40% to 36.94%. Figure 2 shows significant positive correlations among DPPH, ABTS, Fe^{2+} chelation, and hydroxyl radical scavenging activities in raw and autoclaved *Dioclea reflexa* seed flours.

Table 5. Antioxidant activities (%) of autoclaved marble vine *(Dioclea reflexa)* seed flours and control samples.

Samples	DPPH Radical Scavenging Activity (%)	ABTS Radical Cation Scavenging Activity (%)	Ferrous Ion (Fe^{2+}) Chelating Activity (%)	Hydroxyl Radical Scavenging Activity (%)
RLB	79.37 ± 0.05 ^{a}	58.71 ± 0.10 ^{a}	68.91 ± 0.20 ^{a}	61.38 ± 0.22 ^{a}
ALB10	70.30 ± 0.04 ^{c}	50.88 ± 0.11 ^{c}	60.11 ± 0.08 ^{c}	53.46 ± 0.09 ^{b}
ALB30	67.06 ± 0.28 ^{d}	45.74 ± 0.12 ^{e}	53.43 ± 0.09 ^{e}	47.22 ± 0.07 ^{d}
RDB	74.82 ± 0.59 ^{b}	47.22 ± 0.16 ^{d}	65.49 ± 0.09 ^{b}	41.95 ± 0.27 ^{f}
ADB10	70.65 ± 0.18 ^{c}	40.51 ± 0.20 ^{g}	60.54 ± 0.05 ^{c}	33.65 ± 0.03 ^{h}
ADB30	66.32 ± 0.02 ^{d}	38.33 ± 0.27 ^{h}	55.83 ± 0.07 ^{d}	28.55 ± 0.06 ^{i}
RB	73.99 ± 0.57 ^{b}	52.40 ± 0.08 ^{b}	59.25 ± 0.29 ^{c}	51.66 ± 0.08 ^{c}
AB10	69.91 ± 0.25 ^{c}	42.63 ± 0.11 ^{f}	52.37 ± 0.03 ^{f}	43.28 ± 0.02 ^{e}
AB30	61.76 ± 0.15 ^{e}	36.94 ± 0.22 ^{i}	46.30 ± 0.04 ^{g}	37.79 ± 0.03 ^{g}

Values are Mean ± standard deviation. Mean values with the same superscript along the same column are not significantly different at $p < 0.05$. RLB*:* raw light brown; ALB10 and ALB30*:* light brown seeds autoclaved for 10 and 30 min; RDB*:* raw dark brown; ADB10 and ADB30*:* dark brown seeds autoclaved for 10 and 30 min; RB: raw black; AB10 and AB30*:* black seeds autoclaved for 10 and 30 min.

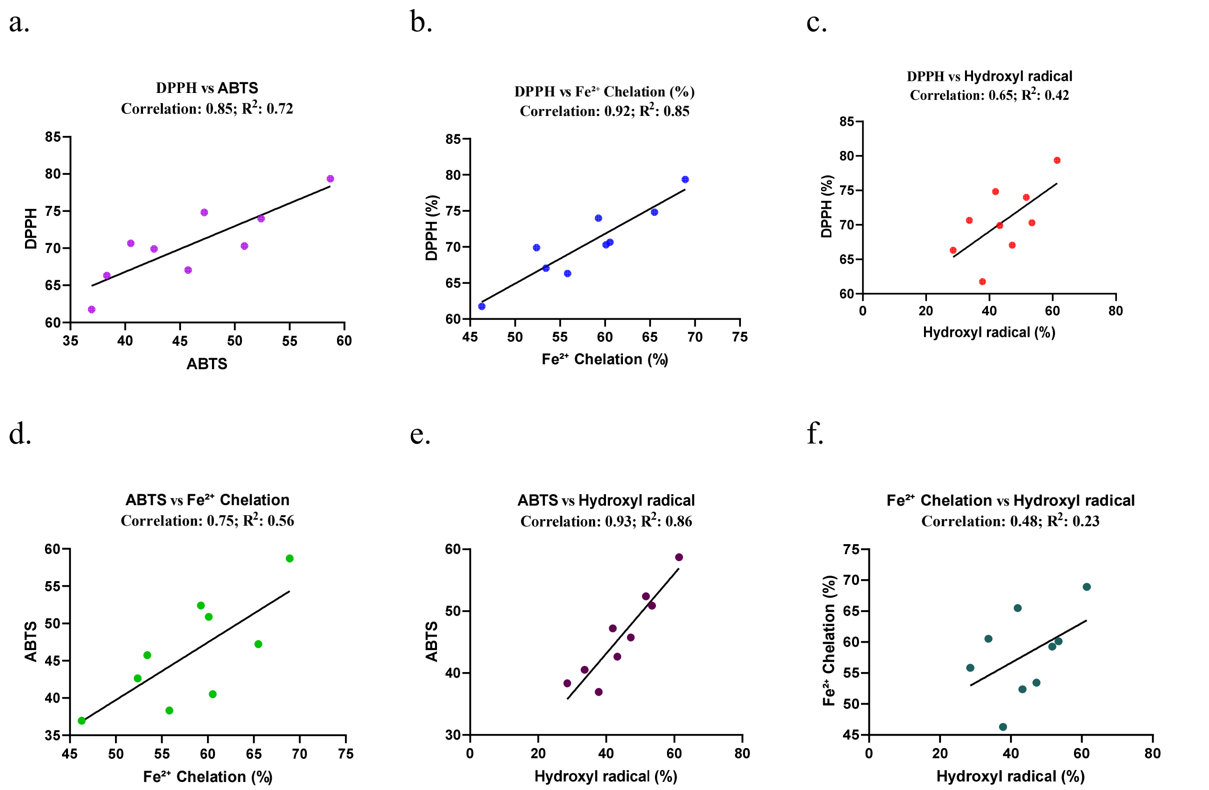

Figure 2. Pairwise correlations among antioxidant activity assays of raw and autoclaved *Dioclea reflexa* seed flours. (**a**) DPPH vs. ABTS radical scavenging activities; (**b**) DPPH radical scavenging activity vs. Fe^{2+} chelating activity; (**c**) DPPH vs. hydroxyl radical scavenging activities; (**d**) ABTS radical scavenging activity vs. Fe^{2+} chelating activity; (**e**) ABTS vs. hydroxyl radical scavenging activities; and (**f**) Fe^{2+} chelating activity vs. hydroxyl radical scavenging activities. RLB: raw light brown; ALB10 and ALB30: light brown seeds autoclaved for 10 and 30 min; RDB: raw dark brown; ADB10 and ADB30: dark brown seeds autoclaved for 10 and 30 min; RB*:* raw black; AB10 and AB30*:* black seeds autoclaved for 10 and 30 min.

3.6. Carbohydrate-Hydrolysing Enzyme Inhibition of Autoclaved Dioclea Reflexa Seed Flours

Autoclaving led to increased α-amylase and α-glucosidase inhibitory activities across all *Dioclea reflexa* seed flour varieties (Table 6). The light brown variety (ALB 30) exhibited the highest inhibition, with values of 86.98% for α-amylase and 68.31% for α-glucosidase, while the raw sample of this variety recorded lower values of 69.99% and 55.17%, respectively. In the dark brown variety, α-amylase inhibition increased from 55.62% in the raw sample to 69.99% after ten minutes of autoclaving and further to 78.78% after thirty minutes. Similarly, α-glucosidase inhibition in the dark brown variety rose from 42.96% in the raw sample to 52.07% and 58.09% after ten and thirty minutes of autoclaving, respectively. The black variety showed a similar trend, with α-amylase inhibition increasing from 60.36% (raw) to 69.88% and 77.53% after ten and thirty minutes of autoclaving, respectively, and α-glucosidase inhibition increasing from 43.68% (raw) to 52.53% and 59.38% under the same conditions.

Table 6. Carbohydrate-hydrolysing enzyme inhibition of autoclaved marble vine *(Dioclea reflexa*) seed flours and control samples.

Samples	α-Amylase (%)	α-Glucosidase (%)
RLB	69.99 ± 0.22 ^{e}	55.17 ± 0.09 ^{e}
ALB10	79.77 ± 0.18 ^{b}	61.33 ± 0.08 ^{b}
ALB30	86.98 ± 0.15 ^{a}	68.31 ± 0.11 ^{a}
RDB	55.62 ± 0.20 ^{b}	42.96 ± 0.11 ^{h}
ADB10	69.99 ± 0.16 ^{e}	52.07 ± 0.31 ^{f}
ADB30	78.78 ± 0.29 ^{c}	58.09 ± 0.09 ^{d}
RB	60.36 ± 0.10 ^{f}	43.68 ± 0.29 ^{g}
AB10	69.88 ± 0.17 ^{e}	52.53 ± 0.11 ^{f}
AB30	77.53 ± 0.13 ^{d}	59.38 ± 0.19 ^{c}

Values are mean ± standard deviation. Mean values with the same superscript along the same column are not significantly different at $p < 0.05$. RLB*:* raw light brown; ALB10 and ALB30: light brown seeds autoclaved for 10 and 30 min; RDB: raw dark brown; ADB10 and ADB30: dark brown seeds autoclaved for 10 and 30 min; RB*:* raw black; AB10 and AB30: black seeds autoclaved for 10 and 30 min.

3.7. Principal Component Analysis

Principal component analysis revealed that the first three principal components explained 91.0% of the total variance in the dataset. The first principal component (PC1) accounted for 48.8% of the variance and was primarily associated with antioxidant indices, mineral content, antinutritional factors, and enzyme inhibitory variables. The second principal component (PC2) explained 29.6% of the variance and was mainly associated with proximate nutritional parameters, including ash, carbohydrate, protein, and energy content, while the third principal component (PC3) accounted for 12.6% of the variance. The PCA biplot (Figure 3) showed clear separation of samples based on seed variety and autoclaving time.

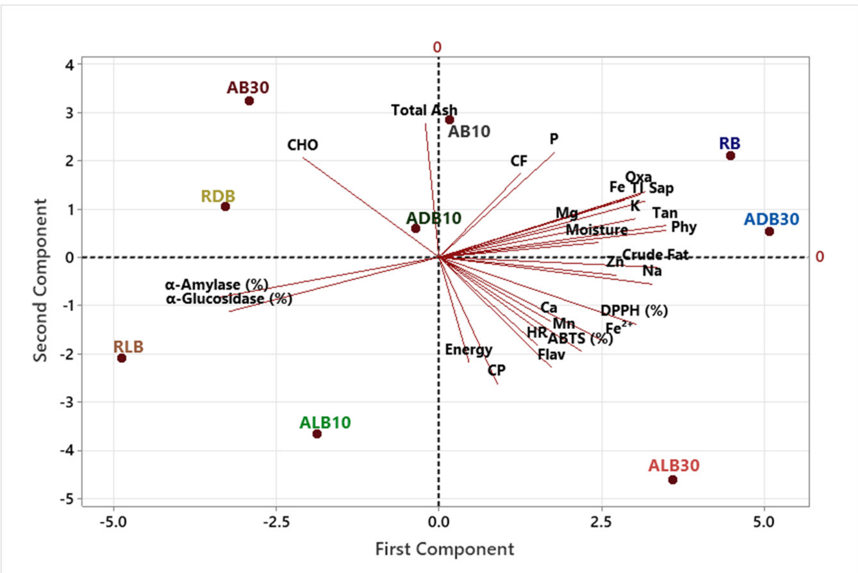

Figure 3. Principal Component Analysis (PCA) biplot showing relationships among nutritional composition, mineral content, phytochemicals, antioxidant activities, and enzyme inhibitory properties of raw and autoclaved

Dioclea reflexa seed flours. CHO: carbohydrate; CP: crude protein; CF: crude fibre; TI: trypsin inhibitor; Oxa: oxalate; Sap: saponin; Tan: tannin; Phy: phytate; Flav: flavonoid; HR: hydroxyl radical scavenging activity (%); Fe^{2+}: Fe^{2+} chelation activity (%). RLB*:* raw light brown; ALB10 and ALB30: light brown seeds autoclaved for 10 and 30 min; RDB: raw dark brown; ADB10 and ADB30*:* dark brown seeds autoclaved for 10 and 30 min; RB*:* raw black; AB10 and AB30: black seeds autoclaved for 10 and 30 min. PC1 and PC2 represent the first two principal components explaining the highest proportion of total variance. Variables positioned closer together indicate positive correlations, while variables located in opposite directions indicate negative associations.

4. Discussion

The proximate composition (Table 1) showed that the crude protein content of flour samples from the marble vine seeds examined in this study declined significantly with increasing autoclaving time. This reduction could be attributed to denaturation and degradation of proteins resulting from the heat and pressure applied during the autoclaving process [9]. The light brown variety consistently exhibited the highest protein levels, with 24.94 g/100 g in the raw sample (RLB) and 21.44 g after 30 min of autoclaving (ALB30), while the black variety had the lowest (16.19 g in RB, 12.56 g in AB30). These values are considerably higher than those reported for roasted light brown (16.84 g to 24.32 g) but lower than the dark brown and black (16.47 g to 29.46 g;17.50 g to 22.79 g) marble vine seeds [3]. These findings suggest that the total protein content of marble vine seed could vary depending on the seed type and processing methods [7]. Moisture content across the samples ranged from 4.52 g to 8.78 g/100 g, with the highest in the raw dark brown seeds (RDB) and the lowest in ALB30. A general decline in moisture was observed with increasing autoclaving time across all seed types, enhancing the potential shelf life of the flour. This trend aligns with findings from Negedu et al. [28], who reported lower moisture in autoclaved castor seeds relative to raw ones. Notably, the moisture content of all the flour samples (4.52–8.78 g/100 g) was well within the safe range (<10 g/100 g) established by NIS [29] for food products. This implies that autoclaving can be beneficial for improving the keeping quality of marble vine seed flours [30]. A marked decrease in fat content of the flour samples was also observed across all varieties with longer autoclaving durations. This supports previous findings by Omotoso et al. [7], Arise et al. [1], and Anyiam et al. [31], which linked heat treatment with lipid loss in leguminous seeds. Fat loss is beneficial in preventing rancidity and off-flavours development in food products. Additionally, energy values ranged from 372.72 kcal to 383.52 kcal/100 g across all varieties and treatments, with the highest in RLB and the lowest in ADB10. Autoclaving slightly reduced energy values across all samples, likely due to concurrent losses in protein and fat. Nonetheless, these values remain higher than those of many other underutilized legumes such as Lima bean, Jack, African yam bean and Mung bean reported by Popoola et al. [2].

Generally, the mineral composition of the flours obtained from marble vine seeds were found to decrease with increasing autoclaving time. This loss of mineral content in autoclaved samples may be attributed to the leaching of minerals during the autoclaving process, as suggested by Farinde et al. [32] in their study on cooked lima bean samples. Interestingly, the potassium content of *Dioclea reflexa* seed flours was higher than values reported for *Mucuna flagellipes* seed flours subjected to soaking, roasting, boiling, and autoclaving [33]. This suggests that *D. reflexa* may serve as a comparatively richer dietary source of potassium among underutilized legumes. Maintaining adequate levels of potassium is essential for regulating fluid balance, supporting healthy nerve function, and facilitating muscle contractions [30,32]. Calcium and Phosphorus are essential minerals that play several biological functions, including nerve transmission, cell signaling, bone formation, blood clotting, and pH regulation [34]. In this study, the calcium content of all the samples dropped significantly after autoclaving, with samples autoclaved for 30 min showing up to 69% (ALB 30) loss. This result is in line with the findings of Yahaya et al. [35], where steaming resulted led to significant reductions in the calcium content of red and cream varieties of Bambara groundnut. Similarly, Phosphorus content also decreased after autoclaving, consistent with reports on lima beans, brebra seeds, and Bambara groundnuts. However, these legumes showed higher phosphorus levels than Dioclea reflexa seed flours in this study [2,35,36]. Overall, the dark brown variety tended to retain higher mineral content post-autoclaving compared to light brown and black varieties. Additionally, increasing autoclaving duration consistently amplified mineral reductions regardless of seed variety. Although minerals are not destroyed by heat, the marked reductions in sodium (97%) and calcium (72%) following autoclaving reflect extensive mineral leaching during autoclaving. In this study, contact with liquid during autoclaving and the removal of hulls likely contributed to the reductions observed [37]. Such leaching can also affect phytate-to-mineral molar ratios and mineral bioavailability. Mineral losses may be mitigated by retaining or upcycling the processing water in food applications, as well as through post-processing fortification, particularly with calcium salts. In addition, process optimization strategies such as reducing autoclaving time, limiting water contact, or employing steam-based treatments have been shown to reduce mineral leaching [8,38]. Future research should include analyses of processing water and by-products to better evaluate mineral loss mechanisms.

Furthermore, the flour samples obtained from the three varieties of marble vine seeds contained various phytochemicals, including saponin, phytate, flavonoid, tannin, oxalate, and trypsin. They are known to possess several health benefits, such as antimicrobial, antidiarrheal, antioxidant, anticancer, and atherosclerosis benefits in humans [36]. However, if the amount of these phytochemicals in food exceeds acceptable limits, they can negatively impact the body's ability to digest and absorb nutrients like protein, vitamins, and minerals. Therefore, their concentration in foods, which can be influenced by processing techniques such as autoclaving, appears to be associated with their effects [15]. In this study, the light brown had the lowest tannin content both before and after autoclaving. The unprocessed flour samples had higher tannin content compared to the autoclaved ones, indicating that autoclaving reduced the levels of tannin in the samples. These findings are consistent with some past reports where autoclaving for about 10 min decreased the tannin content for up to 65%, 65.1%, 71.1%, and 90% in Cowpea, Pigeon pea, Soybean, and guar bean varieties, respectively [10,39,40]. Likewise, the trypsin inhibitors and flavonoid content of the flour declined considerably after autoclaving. For trypsin inhibitors, similar reports on autoclaved mung bean, cooked lima bean, and soaked + autoclaved jack bean, noted around 35%, 96.37%, and 96% loss, respectively [1,32,41]. The decrease in trypsin inhibitors after autoclaving could be as a result of thermal denaturation of their covalent bonds, causing loss of biological activity, and from the pressure applied during the process, which forces water into the seeds and increases their extraction [7,42]. This result agrees with the observation of Wodajo and Emire [42], reporting a decline in flavonoid content of haricot beans after autoclaving. Flavonoids are recognized for their health-promoting effects, especially their ability to bolster immune function and improve vascular health. In terms of phytates, the amount found in all flour samples was below the range of 1000–6000 mg/100 g (equivalent to 10–60 mg/g), which is considered safe for human health [43]. Phytates are a rich source of phosphate and inositol found in plants and can decrease the rate of carbohydrate digestion and lower blood glucose levels [8]. However, they are known for their potential to reduce the bioavailability of minerals like calcium, iron and zinc by forming insoluble complexes that hinder mineral absorption [15]. The values of Phy/Zn molar ratios showed that the absorption of zinc in the body may not be impaired by the phytate levels in ALB10 and ALB30 and the Phy*Ca/Zn for all flour samples were below the threshold that indicates poor zinc bioavailability [44].

The results revealed significant alterations in the phenolic profile of *Dioclea reflexa* seed flours after autoclaving, with effects varying by seed variety (light brown, dark brown, and black) and autoclaving duration (10 and 30 min). Overall, autoclaving increased the concentration of most individual phenolic compounds, consistent with previous reports that thermal processing enhances phenolic extractability by disrupting cell walls and releasing bound phenolics [45]. Catechin was the most abundant phenolic identified and increased significantly across all seed varieties following autoclaving, with the most pronounced increase observed in black seeds, where levels rose from 90.68 mg/kg (RB) to 119.66 mg/kg (AB10). Similar increases were observed for quercetin and chlorogenic acid. These changes are likely attributable to the release of phenolics previously bound within the seed matrix during moist heat treatment, as reported for other legumes and cereals [46–48]. The stacked bar chart (Figure 1) reveals shifts in the relative contribution of individual phenolics following autoclaving. The observed increase in total phenolics was largely driven by a few dominant flavonoids, particularly catechin, rather than a uniform rise across all compounds. The figure also highlights that autoclaving for 10 min maximized phenolic release, while prolonged treatment (30 min) reduced the contribution of several heat-sensitive phenolics, indicating possible thermal degradation. In addition, the black variety exhibited greater phenolic diversity and abundance than the light and dark brown varieties. Despite the increase in extractable phenolics, antioxidant capacity did not increase proportionally. This suggests that higher phenolic content does not necessarily translate into enhanced antioxidant activity. The observed increase in total phenolics is likely due to improved extractability rather than de novo synthesis, a phenomenon widely reported in thermally processed legumes [12,13]. However, the reduction in radical-scavenging activity indicates qualitative changes in phenolic composition. Thermal processing can induce degradation, polymerization, or structural modification of phenolics, thereby reducing the availability of reactive hydroxyl groups required for antioxidant activity [46]. In addition, protein denaturation during heating may promote the formation of phenolic-protein complexes with reduced antioxidant effectiveness. Furthermore, the Folin–Ciocalteu assay may detect Maillard reaction products formed during autoclaving, which can inflate total phenolic values without a corresponding increase in antioxidant activity [49]. Among the varieties studied, black seed samples consistently exhibited the highest phenolic concentrations after processing. This agrees with reports on colored grains and pulses, where darker seed coats are associated with richer phenolic profiles [46]. Consequently, the improved phenolic profile of black seed samples indicates their greater potential as functional ingredients compared to light and dark brown varieties, particularly following appropriate thermal treatment. Autoclaving for 10 min was generally more effective in maximizing individual phenolics in black seeds, especially catechin, quercetin, and chlorogenic acid, whereas prolonged autoclaving (30 min) led to reductions in some compounds. This observation aligns with findings by Niabaho et al. [50], who reported that autoclaving reduced certain polyphenols while promoting the formation of others in brewer's spent grain. Interestingly, although specific flavonoids such as catechin and epicatechin increased after autoclaving, total flavonoid content (reported under antinutritional factors) declined. This discrepancy may reflect differences in analytical methods, as total flavonoid assays estimate a broader range of compounds, some of which may be heat-labile. A similar pattern was reported by Siah et al. [46], who observed that thermal processing altered the phenolic profile of faba beans due to the breakdown of complex polyphenols or the formation of compounds not detected by conventional colorimetric assays. In summary, autoclaving significantly enhanced the phenolic composition of *Dioclea reflexa* seeds, with the extent of these impacts influenced by processing duration and seed variety. Overall, the varietal differences in response to autoclaving may be attributed to inherent differences in seed coat pigmentation, phenolic localization, and overall matrix composition, including protein–phenolic interactions and mineral binding sites [51–53]. Darker-colored legumes often contain more complex and tightly bound constituents that respond differently to thermal processing [3,11,46]

Antioxidants are crucial for maintaining health by defending the body against oxidative stress, a major factor in the development of chronic diseases such as diabetes and hypertension [54]. Our study investigated the effect of autoclaving on the antioxidant properties of *Dioclea reflexa* seed flours, revealing significant differences between raw and autoclaved samples. Across all antioxidant assays; DPPH, ABTS, Fe^{2+} chelation, and hydroxyl radical scavenging: raw samples consistently demonstrated significantly higher antioxidant activity compared to the autoclaved samples. These findings align with previous research indicating variable effects of thermal processing on antioxidant parameters. For instance, autoclaving has been associated with reductions in total phenolic content (TPC) and total flavonoid content (TFC) in mung beans [55]. Similarly, Kataria et al. [56] reported a decline in DPPH and ABTS radical scavenging capacities in teff grain following autoclaving. These reductions are often attributed to the thermal degradation of antioxidant compounds and their potential leaching into cooking water during prolonged heat exposure, leading to a diminished overall antioxidant potential in the processed samples. Conversely, Ajatta et al. [3] utilized response surface methodology to optimize roasting parameters for maximizing the antioxidant and antidiabetic potential of *D. reflexa* seed flours. The study demonstrated that moderate roasting enhanced DPPH and ABTS radical scavenging activities. This increase was attributed to the formation of Maillard reaction products and the release of bound phenolic compounds, both of which contribute significantly to antioxidant capacity [3]. In our study, DPPH radical scavenging activity was consistently high across all *D. reflexa* samples, suggesting the presence of potent hydrogen-donating antioxidants within the seeds [3,57]. Regarding the variations in antioxidant capacity among the three varieties, the light brown seed variety exhibited the highest metal chelating ability, even after autoclaving. The strong chelating capacity observed in all samples, particularly the raw flours, highlights the inherent functional potential of *D. reflexa*. The chelation of metal ions such as Fe^{2+} and Cu^{2+} is a crucial mechanism for preventing the Fenton reaction, thereby limiting the generation of harmful reactive oxygen species [3]. Overall, the light brown variety of *Dioclea reflexa* exhibited the highest overall antioxidant capacity among the studied seed varieties.

The strong positive correlations among DPPH, ABTS, Fe^{2+} chelation, and hydroxyl radical scavenging activities indicate that antioxidants in *Dioclea reflexa* seed flours can function across multiple oxidative systems. Samples with higher radical scavenging activity also exhibited greater metal chelation and hydroxyl radical scavenging capacities, suggesting the presence of compounds capable of acting through multiple antioxidant mechanisms. These findings further support the role of phenolic compounds, which may be released or structurally modified during autoclaving, thereby influencing the overall antioxidant capacity. Nonetheless, variations in correlation strength among the different assays suggest that thermal processing may differentially affect phenolic compounds, leading to variations in their antioxidant effectiveness across mechanisms [58–60].

Furthermore, autoclaving significantly enhanced the α-amylase and α-glucosidase inhibitory activities of *Dioclea reflexa* seed flours, with effects varying by seed variety and processing time. The observed increases in enzyme inhibition suggest that thermal processing can improve the antidiabetic potential of *D. reflexa*, potentially through the release or transformation of bioactive compounds such as phenolics, which are known to inhibit carbohydrate-hydrolyzing enzymes [61]. The result is consistent with previous findings in other leguminous foods that showed varying effect of thermal processing depending on the legume source and processing method adopted [40]. Among the three varieties, the light brown seeds showed the most pronounced response to autoclaving, particularly at thirty minutes, where α-amylase and α-glucosidase inhibition reached 86.98% and 68.31%, respectively. This represents a substantial improvement over the raw sample of the same variety. The dark brown and black varieties also demonstrated increased inhibitory activity with autoclaving, though to a lesser extent than the light brown variety. Inhibition of α-amylase and α-glucosidase is a key strategy in the dietary management of postprandial hyperglycemia. Therefore, the improved inhibitory activity observed in autoclaved *D. reflexa* flours highlights their potential as functional food ingredients for glycemic control [62].

The PCA results highlight strong multivariate associations among antioxidant activity, mineral composition, and bioactive compounds, particularly in autoclaved samples. The clustering of antioxidant indices with phenolic-related variables supports the contribution of phenolic constituents released or structurally modified during autoclaving to functional properties. In contrast, the distinct positioning of α-amylase and α-glucosidase inhibitory activities suggests that enzyme inhibition is influenced by factors beyond total phenolic content, including specific phenolic structures and protein–phenolic interactions. The clear separation of samples according to variety and processing time further confirms that both varietal characteristics and thermal treatment jointly influence the nutritional and functional quality of *D. reflexa* seed flours.

5. Conclusions

Autoclaving significantly influenced the nutritional quality and bioactive properties of *Dioclea reflexa* seed flours, with effects strongly dependent on seed variety and processing time. Protein and mineral contents declined following autoclaving, largely due to denaturation and leaching. However, the light brown variety maintained acceptable zinc bioavailability. In contrast, α-amylase and α-glucosidase inhibitory activities improved following autoclaving, especially the light brown variety. Overall, varietal differences suggest that black-seeded *D. reflexa* has greater potential for phenolic-rich functional applications, whereas light brown varieties may be more suitable for dietary management of postprandial hyperglycemia. These findings highlight the importance of selecting appropriate seed varieties and optimizing autoclaving conditions to maximize the nutritional and functional value of *D. reflexa* seed flours in food applications.

Author Contributions

A.I.A.: investigation; conceptualization; methodology; resources, supervision; validation; writing—review and editing; T.Y.A.: investigation; methodology; data curation; writing—original draft; A.I.I.: investigation, resources; formal analysis; data curation; M.C.C.: investigation, resources; formal analysis; data curation. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

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Use of AI and AI-Assisted Technologies

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