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Nutritional and Phenolic Profile of Early and Late Harvested Amaranth Leaves Grown Under Cultivated Conditions

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Abstract: Amaranth leaves are edible vegetables with high nutritional value that depends on the harvest stage. This study evaluated the nutritional and chemical profile of amaranth leaves harvested at days 65 and 120. Samples were collected, air-dried, and milled to pass through a 1-mm sieve before analysis. Higher ($p < 0.05$) crude protein (CP) and gross energy (GE) were observed in early harvested leaves than late harvested leaves. In addition, late harvested leaves had higher ($p < 0.05$) calcium, magnesium, and sodium contents than early harvested leaves; early harvested leaves had higher ($p < 0.05$) threonine, lysine, and leucine contents than late harvested leaves. Furthermore, early harvested leaves showed higher ($p < 0.05$) rutin, hyperoside, tryptophan, quercetin, and kaempferol rutinoid contents than late harvested leaves. A strong positive correlation was observed between nutrition composition and phenolic compounds. It can be concluded that both early and late harvested amaranth leaves are a promising source of nutrients and phenolic compounds that can help in providing new opportunities for their use in the food and pharmaceutical industries.

Keywords: amaranth leaves; amino acids; harvest stage; minerals

1. Introduction

A trend has been observed recently where consumers are beginning to develop an interest in healthy diets containing plant-based foods such as fruits and vegetables, cereals, legumes, and nuts [1]. These plant-based foods not only play a significant role in improving and maintaining human health, but are also environmentally sustainable [2]. According to a World Health Organization (WHO) report, the majority of developing countries are faced with the challenge of macronutrient deficiencies mostly targeting younger children and pregnant women [3]. Moreover, for any population to maintain healthy behavior, accessibility, availability, and affordability of a variety of foods from either plant or animal origin play a significant role [4]. One identified crop that has the potential to alleviate malnutrition and is readily accessible and affordable is ancient amaranth, which has been receiving renewed attention today. It is regarded as a leafy green vegetable that has the potential of being part of a healthy diet [5,6]. This leafy vegetable is abundant in vitamins, minerals, and fiber but low in calories [7]. Thus, consuming a diet rich in amaranth can offer numerous health benefits including reduced risk of obesity, heart disease, high blood pressure, and mental decline [7,8]. Furthermore, amaranth leaves have high protein content and balanced amino acid composition [9]. Several studies have proven health benefits derived from human beings consuming the amaranth vegetable. Such proven health benefits include assisting in the recovery of severely malnourished children and an increase in the body

mass index of people [10]. However, the amaranth vegetable contains secondary metabolites such as tannins, oxalates, phytates, trypsin inhibitors, saponins, and nitrates, which are reported to reduce the availability of certain minerals such as calcium in the human body [11,12]. However, several methods have been successfully employed to decrease concentrations of these chemicals and make it suitable for feeding [13,14].

The most attractive part of amaranth is its agronomical traits, which include its drought resistant nature and high tolerance to arid conditions. However, prolonged dry periods induce flowering and decrease leaf yields [15]. Efficient use of amaranth leaves as food depends on nutritional profile, which in turn influences the quantity and quality of outputs from animal production systems. The stage of harvest can be used to measure the nutritional profile of crops like amaranth and has shown to affect crop yields and nutritional contents [16]. Moreover, soil conditions, fertilizers, and moisture availability are also known as factors that can affect crop yields and nutritional composition. Harvesting early before the plant reaches its maturity stage or late when the plant has surpassed its maturity age can have a negative effect on the nutritional status of crops [17,18]. This negative effect has shown to occur mostly with protein content because it can be easily affected by the stage of maturity [19]. A decrease in protein normally favors an increase in structural components of plants such as lignin, hemi-cellulose, and cellulose as well as anti-nutritional compounds [17]. An increase in these structural components has been shown to decrease palatability and digestibility, whereas anti-nutritional factors have been reported to inhibit the availability of other nutrients and thus result in a negative effect [11,12,20,21]. It is therefore of paramount importance to determine what stage of harvest is suitable when considering utilizing amaranth leaves for food. Limited information is available on at which stage of growth amaranth leaves can be harvested in order to maximize nutrient availability principally for species grown under cultivated South African conditions. Thus, the core aim of this study was to evaluate the nutritional and chemical profile of amaranth leaves grown under cultivated conditions harvested at day 65 (before the plant reaches maturity) and 120 (after the plant has reached its maturity stage).

2. Materials and Methods

2.1. Harvesting of Amaranth Leaves

Amaranth cruentus (Latin) leaves used in the present study were grown under a controlled field trial in the Northwest Province of South Africa. The mean temperatures in the area are above 22 °C in summer and below 20 °C in winter, and it lies at a latitude of 25.6200°S and longitude 27.9800°E. This variety was grown in September 2019 under dry land conditions that collect a mean annual rainfall of less than 250 mm. Amaranth leaves were harvested in two stages, resulting in an early and a late harvest. The first harvest (early harvest) of leaves was done before the plant reached maturity. The second harvest of leaves was done at day 120 of the cycle, when the plant had reached maturity. The harvested leaves were then independently dried in a well-ventilated laboratory to obtain constant weight and milled into powder through a 1-mm sieve using a hammer mill before being subjected to chemical analyses as described below.

2.2. Chemical Analysis

Proximate analysis for moisture, ash, crude protein (CP, $N \times 6.25$), fat, and starch were carried out according to Association of Official Analytical Chemist (AOAC) [22] method number 942.05. Ground samples of amaranth leaves were oven-dried and weighed. Thereafter, samples in a crucible were ashed in a muffle furnace at 550 °C for 6 h. The ash was acid digested by adding 1 mL 55% (v/v) HNO_3 . After cooling, calcium, magnesium, manganese, zinc, iron, sodium, potassium, copper, sulfur, and phosphorus concentrations were determined by AOAC method 6.1.2 using inductively coupled plasma spectroscopy. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were established according to the methods of Van Soest et al. [23]. The gross energy (GE) contents of the milled samples

and excreta were determined with adiabatic bomb calorimetry. Fat and ether extract lipid content was estimated using TecatorSoxtec.

2.3. Amino Acid Determination

Amino acid separation and detection were performed using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector. One μL of sample/standard solution was injected into the mobile phase, which conveys the derivatized amino acids onto a Waters UltraTax C 18 column ($2.1 \times 50 \text{ mm} \times 1.7 \mu\text{m}$) held at 60°C . Elution of analytes off the column was performed by running a gradient. Analytes eluting off the column were detected by the PDA detector, with each amino acid coming off the column at a unique retention time.

2.4. Phenolic Compounds Determination

The extracts were prepared by using 2 g dry leaf material + 15 mL 50% methanol/1% formic acid in water with ultrasonication for 1 h and standing overnight, followed by centrifugation and transfer of the supernatant to a glass vial ready for the liquid chromatograph-mass spectrometer (LC-MS) analysis. A quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a UPLC (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275°C , and desolvation gas at 650 L/h, and the rest of the MS settings were optimized for best resolution and sensitivity. Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode.

2.5. Statistical Analysis

The collected data were analyzed using a one-way ANOVA of SAS [24] software. The general linear model employed was:

$$Y_{ij} = \mu + SM_i + E_{ij} \quad (1)$$

where Y_{ijk} is the observation of the dependent variable ijk (chemical components of leaves); μ is the fixed effect of the population mean for the variable; SM_i is the stage harvested of leaves ($i = 2$; early and late); and E_{ij} is the random error associated with observation ij , assumed to be normally and independently distributed. Where significant differences were observed, mean separation was done using the Least Significance Difference (LSD) test at the 5% level of significance. Pearson's correlation coefficient was used to determine the relationship between phenolic compounds with chemical composition and mineral compositions. Principal component analysis (PCA) analysis was conducted using PAST version 4.02, a software for scientific data analysis with functions for data manipulation, plotting, univariate, and multivariate statistics analysis.

3. Results

3.1. Proximate Composition of Amaranth Leaves Harvested at Different Stages

The results for the proximate composition of early and late harvested leaves are presented in Table 1. Higher ($p < 0.05$) dry matter (DM) and crude protein (CP) were observed in early harvested leaves (92.65 and 23.23 g/100 g) as compared to those that were harvested late (91.38 and 16.46 g/100 g). The crude fiber (CF) content of late harvested leaves (17.74 g/100 g) was higher ($p < 0.05$) than early harvested leaves (17.14 g/100 g). However, early harvested leaves (15.40, 7.14, 1.95 and 14.50 g/100 g, respectively) had higher ($p < 0.05$) NDF, ADF, acid detergent lignin (ADL), and GE than late harvested leaves (14.45, 6.61, 1.35 and 12.44 g/100 g, respectively). Late harvested leaves (1.52 and 1.11 g/100 g) showed higher ($p < 0.05$) ether extracts (EE) and starch contents than early harvested leaves (1.12 and 0.38 g/100 g). Ash contents of early harvested leaves (21.18 g/100 g) were higher ($p < 0.05$) than late matured leaves (17.70 g/100 g).

Table 1. Proximate analysis composition of early and late harvested amaranth leaves (g/100 g).

Nutrient	Stage of Harvesting		Probability
	EHL	LHL	
DM	92.65 ^a ± 0.73	91.38 ^b ± 0.72	0.0031
CP	23.23 ^a ± 3.91	16.46 ^b ± 0.71	0.0001
CF	17.14 ^b ± 0.35	17.74 ^a ± 0.36	0.0136
NDF	15.40 ^a ± 0.071	14.45 ^b ± 0.071	0.0055
ADF	7.14 ^a ± 0.31	6.61 ^b ± 0.30	0.0173
ADL	1.95 ^a ± 0.35	1.35 ^b ± 0.34	0.0136
GE	14.50 ^a ± 1.19	12.44 ^b ± 1.18	0.0012
EE	1.12 ^b ± 0.24	1.52 ^a ± 0.26	0.0299
Starch	0.38 ^b ± 0.43	1.11 ^a ± 0.45	0.0093
Ash	21.18 ^a ± 2.01	17.70 ^b ± 1.99	0.0004

The results are presented as mean ± standard deviation ($n = 4$). ^{a, b}: Means followed by the same superscript in a row are not significantly different ($p > 0.05$). EHL: Early harvested leaves; LHL: Late harvested leaves.

3.2. Mineral Composition of Early and Late Harvested Leaves

Table 2 presents the mineral contents of amaranth leaves harvested at different stages. Late harvested amaranth leaves had higher ($p < 0.05$) concentrations of calcium, magnesium, and sodium (56,000, 20,350, and 428.21 mg/kg, respectively) than those at the early stage (428.21, 15,295, and 287.87 mg/kg). Concentrations of phosphorus and potassium were higher ($p < 0.05$) in early harvested leaves than in late harvested leaves. Early harvested leaves showed higher ($p < 0.05$) copper, manganese, iron, and zinc (8.95, 583.28, 372.34, and 42.44 mg/kg, respectively) compared to late harvested leaves, which exhibited lower concentrations (3.45, 335.95, 279.95, and 29.95 mg/kg) of these trace minerals.

Table 2. Mineral composition of early and late harvested amaranth leaves (mg/kg).

Nutrient	Stage of Harvesting		Probability
	EHL	LHL	
Calcium	43,287 ^b ± 0.74	56,000 ^a ± 0.075	0.0001
Phosphorus	2044.93 ^a ± 0.06	1371.30 ^b ± 0.07	0.0001
Magnesium	15,295 ^b ± 0.30	20,350 ^a ± 0.31	0.0001
Potassium	14,995 ^a ± 0.13	12,950 ^b ± 0.11	0.0001
Sodium	287.87 ^b ± 0.02	428.21 ^a ± 0.03	0.0001
Copper	8.95 ^a ± 0.071	3.45 ^b ± 0.071	0.0002
Manganese	583.28 ^a ± 0.04	335.95 ^b ± 0.02	0.0001
Iron	372.34 ^a ± 0.03	279.95 ^b ± 0.02	0.0001
Zinc	42.44 ^a ± 0.07	29.95 ^b ± 0.05	0.0001

The results are presented as mean ± standard deviation ($n = 4$). ^{a, b}: Means followed by the same superscript in a row are not significantly different ($p > 0.05$). EHL: Early harvested leaves; LHL: Late harvested leaves.

3.3. Amino Acid Profiles of Amaranth Leaves Harvested at Different Stages

The amino acid composition of early and late harvested amaranth leaves is shown in Table 3 where the results showed that no significant differences ($p > 0.05$) were observed in the histidine, tyrosine, and methionine contents of early and late harvested leaves, even though early harvested leaves showed slightly high contents of the above-mentioned amino acids. Early harvested leaves had higher ($p < 0.05$) arginine, threonine, lysine, and leucine contents (0.90, 0.85, 1.73, and 1.55 g/100 CP respectively), whilst late harvested leaves exhibited lower contents (0.59, 0.58, 1.23, and 1.07 g/100 CP). Late harvested leaves contained higher ($p < 0.05$) valine contents (1.84 g/100 CP) as compared to early harvested leaves (1.51 g/100 CP). No significant difference ($p > 0.05$) was observed in glycine, isoleucine, and phenylalanine contents across all the leaves irrespective of the harvesting stage. However, early

harvested leaves had higher ($p < 0.05$) serine, aspartic acid, glutamine, alanine, and proline contents (0.90, 2.16, 2.94, 1.27, and 0.87 g/100 CP, respectively) than late harvested leaves (0.60, 1.36, 1.78, 0.92, and 0.62 g/100 CP, respectively).

Table 3. Amino acid composition of early and late harvested amaranth leaves (g/100 g crude protein, CP).

	Stage of Harvesting		Probability
	EHL	LHL	
Essential Amino Acids			
Histidine	0.29 ^a ± 0.07	0.22 ^a ± 0.06	0.541
Arginine	0.90 ^a ± 0.19	0.59 ^b ± 0.18	0.033
Threonine	0.85 ^a ± 0.06	0.58 ^b ± 0.07	0.035
Lysine	1.73 ^a ± 0.29	1.23 ^b ± 0.27	0.007
Tyrosine	0.52 ^a ± 0.13	0.35 ^a ± 0.14	0.289
Methionine	0.34 ^a ± 0.08	0.24 ^a ± 0.07	0.271
Valine	1.51 ^b ± 0.19	1.84 ^a ± 0.18	0.008
Leucine	1.55 ^a ± 0.28	1.07 ^b ± 0.27	0.011
Non-Essential Amino Acids			
Serine	0.90 ^a ± 0.18	0.60 ^b ± 0.17	0.026
Glycine	0.94 ^a ± 0.13	0.73 ^a ± 0.11	0.116
Aspartic acid	2.16 ^a ± 0.47	1.36 ^b ± 0.46	0.003
Glutamine	2.94 ^a ± 0.67	1.78 ^b ± 0.65	0.001
Alanine	1.27 ^a ± 0.21	0.92 ^b ± 0.23	0.027
Proline	0.87 ^a ± 0.16	0.62 ^b ± 0.15	0.054
Isoleucine	0.83 ^a ± 0.13	0.57 ^a ± 0.10	0.064
Phenylalanine	0.66 ^a ± 0.11	0.49 ^a ± 0.09	0.120

The results are presented as mean ± standard deviation ($n = 4$). ^{a,b}: Means followed by the same superscript in a row are not significantly different ($p > 0.05$). EHL: Early harvested leaves; LHL: Late harvested leaves.

3.4. Phenolic Compounds and Pearson's Correlation

The phenolic compounds of early and late harvested amaranth leaves are shown in Table 4. Early harvested leaves showed significantly higher ($p < 0.05$) contents of rutin, hyperoside, tryptophan, quercetin 3-O-rhamnosyl-glucoside, and kaempferol rutinoside (1222.79, 206.45, 87.77, 79.31 and 188.80 mg/kg, respectively) as compared to late harvested leaves (1041.92, 130.52, 37.62, 73.03, and 164.00 mg/kg, respectively).

Table 4. Phenolic compounds of early and late harvested amaranth leaves (mg/kg).

Phenolic Compound	Stage of Harvesting		Probability
	EHL	LHL	
Rutin	1222.79 ^a ± 104.42	1041.92 ^b ± 104.40	0.0001
Hyperoside	206.45 ^a ± 43.84	130.52 ^b ± 43.83	0.0001
Tryptophan	87.77 ^a ± 28.95	37.62 ^b ± 28.93	0.0001
Quercetin	79.31 ^a ± 3.66	73.03 ^b ± 3.64	0.0001
3-O-rhamnosyl-glucoside	188.80 ^a ± 14.89	164.00 ^b ± 14.86	0.0001
Kaempferol rutinoside			

The results are presented as mean ± standard deviation ($n = 4$). ^{a,b}: Means followed by the same superscript in a row are not significantly different ($p > 0.05$). EHL: Early harvested leaves; LHL: Late harvested leaves.

The results on the correlation between phenolic compounds, macro-minerals, and chemical composition are presented in Table 5. This shows that there is a negative correlation between calcium (Ca) and magnesium (Mg) with rutin (Rut), Hyperoside (Hyp), tryptophan (Try), quercetin 3-O-rhamnosyl-glucoside (Que) and kaempferol rutinoside (Kru). A strong positive relationship was

detected between manganese (Mn) and potassium (K) with Rut, Hyp, Try, Que, and Kru, whilst a negative association between Mn and K with Mg and Ca was observed. A negative correlation was seen between sodium (Na) and Rut, Hyp, Try, Que and Kru, phosphorus (P) and CP showed a strong positive correlation with Rut, Hyp, Try, Que, and Kru. Moreover, P and CP showed a strong correlation with Mn and K. However, P and CP showed a negative correlation with Ca, Mg and Na. There was a negative correlation between CF and Rut, Hyp, Try, Que, and Kru. However, minerals yielded a positive correlation between CF and Ca, Mg and Na; whilst a negative relationship was observed between CF and Mn, K, P, and CP. A positive correlation was observed between NDF, ADF and Rut, Hyp, Try, Que, and Kru. However, a negative association was observed between NDF and ADF with Ca, Mg, and CF. Moreover, minerals indicated a positive correlation between NDF and ADF with Mn, K, P, and CP. A positive correlation was observed between ADL with Rut, Hyp, Try, and Que. In addition, a positive correlation was exhibited between ADL with Mn, K, P, and CP minerals. Nevertheless, a negative correlation was observed between Ca, Mg, Na, and CF.

Phenolic compounds in early and late harvested leaves are displayed in the stacked bar chart (Figure 1). All compounds (rutin, tryptophan, hyperoside, quercetin 3-O-rhamnosyl-glucoside, and kaempferol rutinoside) were displayed in both early and late harvested leaves, with clear indication of the abundance of rutin followed by kaempferol rutinoside, hyperoside, quercetin 3-O-rhamnosyl-glucoside, and tryptophan. However, early harvested leaves showed more content of all the compounds than late harvested leaves (Table 4; Figure 1).

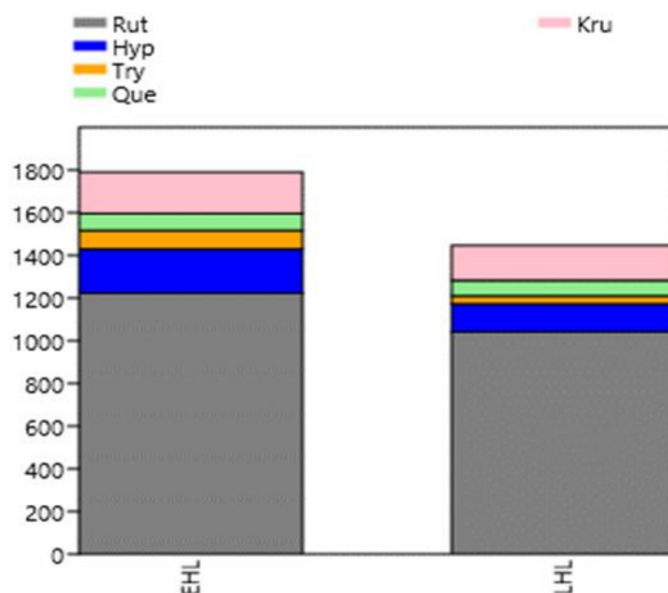


Figure 1. Stacked bar graph of the phenolic compounds (mg/kg) present in amaranth leaves (LHL: late harvested leaves; EHL: early harvested leaves). Rut: rutin, Hyp: hyperoside, Try: tryptophan. Que: quercetin 3-O-rhamnosyl-glucoside, Kru: kaempferol rutinoside.

Table 5. Pearson correlation matrix for phenolic compounds and macro-minerals and chemical composition of amaranth leaves.

	Rut	Hyp	Try	Que	Kru	Ca	Mg	Mn	K	Na	P	CP	CF	NDF	ADF	ADL
Rut	1															
Hyp	1 ***	1														
Try	1 ***	1 ***	1													
Que	1 ***	1 ***	1 ***	1												
Kru	0.962 *	0.962 *	0.962 *	0.962 *	1											
Ca	−1 ***	−1 ***	−1 ***	−1 ***	−0.96 *	1										
Mg	−1 ***	−1 ***	−1 ***	−1 ***	−0.96 *	1 ***	1									
Mn	1 ***	1 ***	1 ***	1 ***	0.962 *	−1 ***	−1 ***	1								
K	1 ***	1 ***	1 ***	1 ***	0.962 *	−1 ***	−1 ***	1 ***	1							
Na	−1 ***	−1 ***	−1 ***	−1 ***	−0.96 *	1 ***	1 ***	−1 ***	−1 ***	1						
P	1 ***	1 ***	1 ***	1 ***	0.962 *	−1 ***	−1 ***	1 ***	1 ***	−1 ***	1					
CP	1 ***	1 ***	1 ***	1 ***	0.964 *	−1 ***	−1 ***	1 ***	1 ***	−1 ***	1 ***	1				
CF	−0.99 **	−0.99 **	−0.99 **	−0.99 **	−0.92	0.986 **	0.986 **	−0.99 **	−0.99 **	0.987 **	−0.99 **	−0.98 *	1			
NDF	0.995 **	0.995 **	0.995 **	0.995 **	0.977 *	−0.99 **	−0.99 **	0.995 **	0.995 **	−0.99 **	0.995 *	0.996 **	−0.96 *	1		
ADF	0.983 *	0.983 *	0.983 *	0.983 *	0.981 *	−0.98 *	−0.98 *	0.983 *	0.983 *	−0.98 *	0.983 *	0.985 *	−0.94	0.997 **	1	
ADL	0.986 **	0.986 **	0.986 **	0.987 **	0.98	−0.99 **	−0.99 **	0.986 **	0.986 **	−0.99 **	0.986 **	0.989 **	−0.95 *	0.998 **	1	1

Ca: calcium, Mg: magnesium, K: potassium, Na: sodium, P: phosphorus, Rut: rutin, Hyp: hyperoside, Try: typtophan, Que: quercetin 3-O-rhamnosyl-glucoside, Kru: kaempferol rutinoside. * indicate a significant correlation ($p < 0.05$); ** indicate a highly significant correlation ($p < 0.01$) *** indicate a very highly significant correlation ($p < 0.001$).

Figure 2 is a loading plot of the phenolic compounds and their percentage contribution to the total variations in amaranth leaves. This confirms that early harvested leaves indeed contain more phenolic compounds, also as previously shown in Figure 1. Total variability among early and late harvested leaves was mainly due to variations in rutin, hyperoside, tryptophan, and kaempferol rutinoside (Figure 2; Table 6).

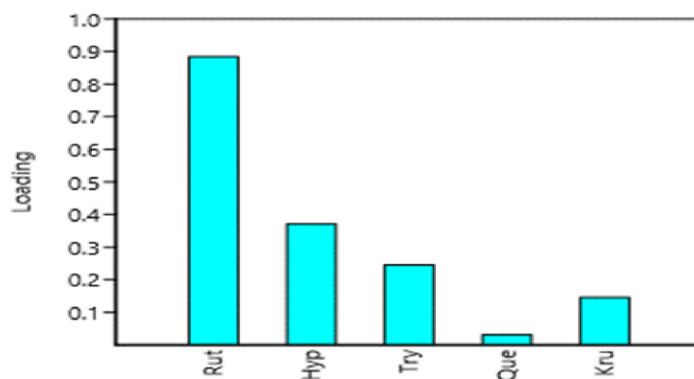


Figure 2. Loadings plot of the phenolic compounds and their percentage (%) contribution to the total variations in amaranth leaves. Rut: rutin, Hyp: hyperoside, Try: tryptophan. Que: quercetin 3-O-rhamnosyl-glucoside, Kru: kaempferol rutinoside.

Table 6. Principal component analysis (PCA) of the phenolic compounds showing their percentage contribution to the total variations.

Principal Component 1 (PC 1)	
Rutin	0.883360
Hyperoside	0.370870
Tryptophan	0.244940
Quercetin 3-O-rhamnosyl-rhamnosyl-glucoside	0.030672
Kaempferol rutinoside	0.145560
Eigenvalue	20961.1
% variance	
Cumulative %	100

PCA of the phenolic compounds in amaranth grains had an eigenvalue of 20,961.1, which is more than 1 (Table 6). Eigenvalues that were more than 1 were seen as significant and component loadings that were larger than ± 0.30 were considered meaningful. Based on this information, the results of this study confirm that variations among early and late harvested leaves were caused by a few flavonoids, mainly rutin, hyperoside, tryptophan, and kaempferol rutinoside.

4. Discussion

Leafy vegetables such as amaranth are promising crops for alleviating poverty and malnutrition in underdeveloped countries [25]. Therefore, understanding its detailed nutritional contents and phenolic compounds should be of outmost importance. Evaluating the agronomic characteristics such as the appropriate stage of harvest or maturity is equally important [16]. The present study presented high DM and CP in early harvested leaves as compared to late harvested leaves. This echoes the findings of Sarmadi et al. [26] and Ma et al. [16], who stated that as the amaranth plant reaches maturity, CP and DM contents decrease. The decrease in CP and DM of late harvested leaves is associated with an increase in structural components of plants as well as phenolic compounds. However, the CF content of late harvested leaves was slightly higher than of early harvested leaves; similar observations were reported by Ma et al. [16]. The increase in the CF contents of late harvested leaves might be due to an increase in lignin, hemicellulose and cellulose responsible for fibrous tissues that maintain plant

structure as it grows [27,28]. Unpredictably, early harvested leaves showed higher NDF, ADF and ADL and GE than late harvested leaves. The outcomes of the current study are contrary to those of Abbasi et al. [29], Hue et al. [30], Ma et al. [16], and Sebola et al. [19], who found an increase in NDF and ADF as amaranth reaches its advance growth. The higher the NDF, ADF, ADL, and GE contents in this study were not expected because during the early growth stage, the plant has not reached its rigidity stage, which is supported by structural components rich in fibers such as NDF, ADF, and ADL and favorable amounts of GE. Moreover, late harvested leaves displayed high EE and starch contents than early harvested leaves. The results of the present study are contrary to the results of He and Corke [31] and Peiretti et al. [19], who observed higher contents of lipids in young leaves. However, high starch content in late harvested leaves was expected because starches are part of non-sugars that give the plant its rigidity, specifically at later stages of growth [31,32].

The findings of the present study further indicate that early harvested leaves can be used as a protein source in diets for growing children, whereas late harvested leaves can be included in diets for adults. The protein requirements in human diet range between 0.66 and 0.69 g/kg [3,33–35]. In the present study, early harvested amaranth leaves were able to supply these requirements.

In the current study, late harvested amaranth had higher concentrations of calcium, magnesium, and sodium than early harvested leaves. These findings concur with the reports of Modi [36], where calcium and magnesium are important constituents of bones and teeth, with their deficiency symptoms presenting in the form of rickets in children and osteomalacia in adult human beings [37]; whereas sodium is known to be a constituent of common salt and serves as osmotic regulation of body fluids [38]. Early harvested leaves exhibited high concentrations of phosphorus, potassium, copper, manganese, iron, and zinc than late harvested leaves. This was expected, since matured leaves have been reported to be rich in anti-nutritional factors known to decrease the bioavailability of minerals [39]. Thus, both early and late harvested leaves can be utilized for consumption, as they can supply the necessary requirements.

The results of the present study showed no variation in the histidine, tyrosine, and methionine contents of early and late harvested leaves, although early harvested leaves exhibited a slightly numerical increase in the above-mentioned amino acids. Early harvested leaves showed high arginine, threonine, lysine, and leucine contents, whereas late harvested leaves exhibited lower contents. Arginine, threonine, lysine, and leucine are essential amino acids that cannot be synthesized by the body and therefore must be supplied in the diet [40].

Late harvested leaves showed high valine, which is part of essential amino acids, contents compared to early harvested ones. Valine helps in repairing damaged tissues, regulating blood levels and promoting normal growth [41]. Non-essential amino acids can be produced by humans even if they are not supplied in their diets. Early harvested leaves had higher serine, aspartic acid, glutamine, alanine, and proline contents than late harvested leaves. These values found in the present study were lower than the values reported by Akubugwo et al. [42]. This might be because their amaranth plant was grown in different climatic conditions. However, both early and late harvested leaves in this study showed appreciable amounts of both essential and non-essential amino acids.

Amaranth leaves are known to have high concentrations of phenolic compounds as the growth stage advances. However, in the present study, early harvested leaves showed significantly higher contents of rutin, hyperoside, tryptophan, quercetin, and kaempferol rutinoside when compared to late harvested leaves. Rutin, hyperoside, tryptophan, quercetin, and kaempferol rutinoside are flavonoids widely known to be the active phytochemical found in various vegetables and fruits [43,44]. There is growing evidence that these flavonoids can be used in human health with antioxidant, antiradical, estrogenic, anti-inflammatory, antiviral, anti-tumoral, anti-diabetic, and cytotoxic activities [45–47]. In the present study, high contents of these flavonoids in early harvested leaves were not expected because these are known to occur more in the stalks, upper parts of well-developed leaves, and sometimes as pigments in fruits [43]. The results of the present study agreed with the results of Karamać et al. [46], who reported high contents of phenolic compounds in young amaranth leaves. These authors stated that the reason

why young leaves have higher phenolic compounds than matured leaves might be that they need these compounds for grain fill stages. Phenolic compounds are known to have antioxidant properties that help in preventing cancer, aging, diabetes, and cardiovascular diseases [7,8]. Thus, early harvested leaves can be suitable for human and livestock use compared to late harvested leaves.

A negative relationship between Ca, Mg, and Na with Rut, Hyp, Try, Que, and Kru was observed. This indicates that as Ca and Mg increase, phenolic compounds decrease. A strong positive relationship was observed between Mn, K and *p* and Rut, Hyp, Try, Que and Kru. Thus, as the level of Mn, K, and P increases, the level of Rut, Hyp, Try, Que, and Kru increases. This concurs with the report of Santos et al. [47], who stated that nutrient availability in plant leaves is related to levels of phenolic compounds present in plant tissues. These results show that flavonoid compound production is dependent on Mn, K, and P contents [45,46]. Moreover, Mn, K, and P are responsible for muscle construction, nerve signaling, and cellular enzyme activity [1,46]. The concentration of flavonoids depends on various factors such as environment, stage of growth, biotic and abiotic stresses, and genotype [47]. With regard to chemical composition, a positive relationship was observed between CP, NDF, ADF and Rut, Hyp, Try, Que and Kru. Thus, as CP, NDF, and ADF contents increase Ru, Hyp, Try, Que, and Kru contents also increase. This result showed that flavonoid compound production in plants also depends on the chemical composition of the plant [48,49]. However, a negative relationship was observed between CF and Ru, Hyp, Try, Que, and Kru. Thus, as CF contents increase, Rut, Hyp, Try, Que, and Kru contents decrease. This is good because if phenolic compounds are in excess quantities, they have the ability to decrease the bioavailability of nutrients to humans [21].

PCA of the phenolic compounds in amaranth leaves has an eigenvalue of more than 1. According to Hair et al. [50], eigenvalues that are more than 1 are seen as significant and component loadings that are larger than ± 0.30 are considered meaningful. Based on this information, the results of this study confirmed that variations among early and late harvested leaves were caused by a few flavonoids, mainly rutin, hyperoside, tryptophan, and kaempferol krutinoside.

5. Conclusions

The results of the present study indicate that both early and late harvested leaves can be a good protein source for young children and adults. Moreover, late harvested leaves can be used as energy sources, since they displayed lower amounts of ADF, NDF, and ADL because they can easily be digested than early harvested leaves that have high contents of ADF, NDF, and ADL. Both early and late harvested leaves contain significant amounts of minerals and amino acids. Early harvested leaves can be recommended as a good source of phenolic acids to aid in fighting cardiovascular diseases in human beings. Conversely, the content of flavonoids (mainly rutin) increased with the growth cycle, and these compounds could be primarily responsible for the antioxidant activity of mature leaves. It is the finding of this study that both early and late harvested amaranth leaves are suitable for human consumption.

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Abbreviations

AOAC	Association of Official Analytical Chemist
LSD	Least Significance Difference
LC-MS	Liquid Chromatograph-Mass Spectrometer
QTDf	A quadrupole time-of-flight
DM	dry matter
CP	crude protein
CF	crude fiber
NDF	neutral detergent fiber
ADF	acid detergent fiber
ADL	acid detergent lignin
GE	gross energy
EE	ether extracts
Ca	calcium
Mg	magnesium
K	potassium
Na	sodium
P	phosphorus
Rut	rutin
Hyp	hyperoside
Try	tryptophan
Que	quercetin 3-O-rhamnosyl-glucoside
Kru	kaempferol rutoside

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