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RESEARCH ARTICLE

The Impact of Varying Stages of Maturity on the Chemical Composition and Antioxidant Activity of Yacon (*Smallanthus sonchifolius*) Leafy Vegetable

Elizabeth Mwongeli Simon*, Monica Wanjiku Mburu, and Richard Kipkorir Koskei

ABSTRACT

Yacon tubers are of global interest due to their prebiotic potential while yacon leaves contain phenolic compounds with potential health benefits. In Kenya, yacon is a newly introduced root crop that is of interest to consumers; however, their main challenge to consumption is determining the appropriate time to harvest the leaves. The chemical makeup of yacon leaves varies depending on the planting site, growing season, harvest time, and growth stages. The current research determined the impact of varying stages of maturity on the chemical composition and antioxidant potential of yacon leaves grown in a farm at Naromoru, Kenya. The leaves were harvested at 2, 3, 4, and 5 months of growth, then subjected to oven drying and stored at 4 °C awaiting analysis. The DPPH assay was used to measure radical scavenging activity, and the Folin–Ciocalteu method was followed in the determination of total phenolic content. Chemical composition was done by Kjedahl method (Crude protein), Soxhlet method (crude fat), and oven drying (moisture content). From the results, the level of maturity significantly ($p < 0.05$) influenced the levels of crude protein, fat and carbohydrates, TPC, and antioxidant activity in the harvested yacon leaves. The crude protein level was highest (28.45%) for the 2nd month of harvest and was lowest in the 5th month (16.75%), while crude fat increased from 3.51% in the 2nd month to 4.20% in the 5th month. Carbohydrates increased from 46.01% to 56.65% in 2nd month to the 5th month, respectively. Both the TPC and antioxidant activity decreased significantly ($p < 0.05$) as the leaves matured. TPC was highest in the 2nd month of harvest and lowest in the 5th month of harvest, 439.33 and 81.00 mgGAE/100 g, respectively. Thus, harvesting yacon leaves at the early stages of growth is crucial to fully benefit from the phytochemical and nutritional composition of the leaves.

Keywords: Antioxidant activity, Proximate composition, Total phenolic compounds, Yacon leaves.

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Institute of Food Bioresources Technology, Dedan Kimathi University of Technology, Kenya.

****Corresponding Author:***
e-mail: mwongeliz2015@gmail.com

1. Introduction

Food culture, eating practices, and dietary habits drive growing public and planetary health concerns over a maintainable supply of food [1]. Worldwide, there is a growing trend in the consumption of plant-based foods that have beneficial effects on physiological and biochemical benefits. According to Charles Boliko [2], hunger is on the rise in most African countries, with the occurrence of undernourished individuals being nearly at 20%. Hunger is not necessarily characterized by inadequate calories but also micronutrient deficiencies [3]. The micronutrient crisis is caused by inadequate intake of proteins, minerals, and microelements, and this has been a public health concern in many countries in sub-Saharan Africa [4] hence buttressing the need for urgent medical and nutrition intervention [5].

Daily consumption of phytonutrient-dense green vegetables can reduce the incidences of cardiovascular diseases, chronic diseases, cancer and boosting immunity [4]. Prevention of protein-energy and micronutrient malnutrition amongst the high-risk populations, therefore, requires creative nutrition-based strategies that promote health. They include dietary diversification [6], [7] and valorization of underutilized crop species such as Yacon (*Smallanthus sonchifoli*us) [8], [9]. Through these strategies, widely consumed staples such as cereals and pulses in these developing countries could be optimized to deliver proteins, micronutrients, and bioactive compounds [10].

Yacon, scientifically known as *Smallanthus sonchifolius* is a South American tuber which is now consumed in various nations, including China, Japan, and South Korea, but it is still a relatively new product on the world market [11]. Both the roots and leaves of yacon are utilized as food because they contain significant amounts of bioactive compounds [12]. These include protocatechuic, chlorogenic, caffeic, and ferulic acids, which make the yacon herbal infusion have antidiabetic and antioxidant properties [13] and have physiological advantages above and beyond simple dietary requirements to lower the risk of chronic diseases [14]. Yacon tubers and leaves are used to make a variety of goods, including syrup, powder, and herbal tea [15]. The yacon leaves have been associated with a number of benefits when taken as a traditional tea, which offers antidiabetic benefits in Asian countries [16]. According to De Andrade *et al.* [16], dried yacon leaves have benefits such as phytochemical activities like antimicrobials, anti-inflammatory and anti-oxidant activities. Moreover, aqueous yacon leaves extracts have been studied in rats and have proven to be safe substance in diabetes treatment in rats as well as proving to have the hypoglycemic effect [17].

Variation in the chemical composition of the yacon leaves is brought about by differences in planting location, growing season, and time of harvest [13]. As plants advance in maturity, the protein content in their leaves declines, whereas the amount of physiologically active components, indigestible structural polysaccharides, and antioxidants rise [18]. Also, fibers develop in the leaves, rendering them less palatable and bitter due to the disposition of anti-nutrients and other compounds [19].

Consuming yacon vegetables at their optimal stage of growth will offer the essential dietary ingestion and maximize food security in Kenya. However, there is currently limited data available regarding the nutritional worth of yacon vegetables that are harvested at different stages of maturity. This research aimed to determine the variation of proximate composition, overall phenolic compounds, and antioxidant activity in yacon leaves harvested at different levels of maturity.

2. Materials and Methods

2.1. Materials

Yacon leaves were acquired from a farm at Naromoru, 0° 10′ 3.22″ N, 37° 1′ 16.64″ E. The leaves were harvested randomly after the 2^{nd} month, 3^{rd} month, 4^{th} month, and 5^{th} month of growth.

2.2. Sample Preparation

The harvested leaves were first sorted out to remove surface dirt and then washed with clean, running tap water. The washed leaves were left to dry at room temperature for one and a half hours. Then they were later spread out on trays and subjected to drying in an oven set at 50 °C for 48 hours. The dried leaves were then ground using a mill into fine flour. The ground leaf samples were kept in airtight storage bags and kept at 4 °C, awaiting further analysis.

2.3. Phenolic Compounds Extraction

Phenolic acids were extracted from the yacon leaves using a technique described by [20]. In brief, 100 mg of powdered leaf samples were added into 20 ml amber EPA vials and then extracted with 10 ml methanol in water (60:40 v/v). The mixtures were vortexed for 30 seconds at 30-minute intervals. Extraction was performed at 25 °C for 18 hours in the dark with constant stirring. The mixture was decanted into a volumetric flask after centrifuging it for 10 minutes at 3000 rpm and at 25 °C. Re-extraction of the residue was done twice for 10 minutes using 5 ml volume of solvent and centrifuged as above. The supernatants obtained were put together and then filtered using filter papers (Whatman No. 1). The methanol was evaporated in a rotary evaporator at 35 °C, and the aqueous portion was used for further analysis.

2.4. Analysis of Proximate Composition

The moisture content, crude ash, crude fat, crude protein as well as crude fiber of the yacon leaves were ascertained using the standard procedures by the Association of Official Analytical Chemists [21].

2.4.1. Moisture Content Determination

Determination of moisture content was done using the oven drying method at 105 °C [21]. About 2 g of the dried leaves were weighed into a labeled and dried moisture dish. The samples were transferred to the oven for 3.5 hours at 105 °C. Thereafter, a desiccator was used to cool the dried samples, and they were weighed. The results were computed as follows:

Moisture content (%) = (*Dry weight* − *Empty weight*)/*Sample wet weight* × 100

2.4.2. Crude Ash Determination

Determination of the crude ash content was done using an electric muffle furnace and using the incineration method (Shimadzu KL-420, Japan). 2 g of samples were burned at 500 °C–600 °C for 6 hours in the muffle furnace to constant weight [21]. After the set time elapsed, the samples remained in the desiccators for cooling, and then their weights were taken. The results were then put across as a percentage of the sample.

2.4.3. Crude Fat Determination

Determination of crude fat content started by extracting fat from 5 g of yacon leaves samples for 6 hours. The determination followed the Soxhlet extraction method with petroleum ether as the solvent. 5 g of the samples were weighed and then transferred to a thimble, then the oil was extracted utilizing petroleum ether (40 °C–60 °C) for 6 hours [21]. The extract acquired was dried in an oven at 105 °C for 30 minutes to distill off the solvent, then allowed to cool in a desiccator, then weighed. Crude fat content was presented as a percentage of the sample.

2.4.4. *Crude Protein Determination*

The crude protein content determination in the yacon leaves was done using the Kjeldahl method described in AOAC [21], 6.25 was the conversion factor used to compute the crude protein amounts from the nitrogen released. A sample of 0.3 g was weighed into the Kjeldahl flask then digested using 10 g copper sulphate, 25 ml of sulphuric acid and 10 g potassium sulphate in a heating block at 370 °C–400 °C for 60–90 minutes or till the contents in the flask became clear. 400 ml of distilled water was added, followed by 80 ml of 40% sodium hydroxide, and the mixture was heated to release ammonia. A solution of boric acid (40 mg/L) containing an indicator composed of bromosocresol green (0.5 g) and 0.1 ml of methyl red dissolved in 100 ml of ethanol at 95% was used to collect the ammonia. Heating was continued until the distillate collected amounted to 50 ml. The boric acid mixture was titrated against 0.1 mg hydrochloric acid until the end point was reached (persisting faded pink color). The blank sample contained the reagents only where the value of the titrate was deducted from the value of the sample.

The protein content was computed using the equation below:

$$\%Protein = \frac{[V_1 - V_0] \times c \times 2.8 \times 6.25}{Weight\ of\ sample}$$

where V_1 is the volume of sulphuric acid used in tests portion, V_0 is the volume of sulphuric acid used in blank test and c is the concentration of sulphuric acid used.

2.4.5. *Crude Fibre*

The crude fibre content determination was done using Hennenberg-Stohmann's technique [21]. I g of the sample was weighed into a crucible with addition of filtering aid. Sequential digestion of yacon leaves samples was done using 1.25% H_2SO_4 and 1.25% NaOH using the fiberglass. Oven drying of the sample in a crucible was done for 5 hours at 105 °C and ashed in a muffle furnace set at 550 °C for 16 hours. The crucibles were left to cool in a desiccator, weighed and the contents of fibre were calculated and recorded. Crude fibre was then presented as percentage of the sample.

2.5. *Total Phenolic Compounds (TPC)*

The Folin-Ciocalteu reagent was used to ascertain the total phenolic compounds in triplicate. 0.5 ml of the extract acquired in Section 2.3 above was put into 0.5 ml of Folin-Ciocalteu solution (20%) and 0.5 ml of sodium carbonate solution (7.5%). After that, the mixture was vortexed and allowed to sit at room temperature for half an hour. The absorbance was read in spectrophotometer at 760 nm. Analytical curve of gallic acid was utilized to measure the phenolic compounds. Presentation of the results was done in mg GAE/g (milligrams of gallic acid equivalents per gram of sample).

2.6. *Determination of Antioxidant Activity*

Spectrophotometry was used to determine the antioxidant activity using the DPPH radical method (1,1-diphenyl-2-picrylhydrazyl). 20 ml of acetone solution at 70% concentration was added to 2 g of powdered yacon leaves. Mixing of the suspension at 180 rpm for 2 hours, then centrifuged for 5 minutes at 2790 g. The supernatant was then moved to an amber bottle and kept in a freezer at -18 ± 1 °C, awaiting analysis. In a light protected test tube, an addition of 100 ml of the extract was done to methanol (1.5 ml) solution of DPPH 0.1 mM and vortexed for a duration of 30 seconds. The solution was left to stand for 30 minutes. Then, a spectrophotometer was used to read the solution's absorbance (Thermo Scientific, Evolution 606, USA) at 517 nm. The results were presented as mmol Trolox equivalent/g sample.

2.7. *Data Analysis*

The data acquired was passed through one-way analysis of variance (ANOVA) using Minitab Release 18 Software (Minitab Inc., Pennsylvania, USA). Means were separated using Fisher LSD at 5% level of significance ($p < 0.05$). The analyzed data were then presented as means ± standard deviation (SD).

3. Results and Discussion

3.1. *Proximate Composition of Yacon Leaves Harvested at Different Stages of Growth*

The dried leave samples of yacon harvested at different levels of maturities were analyzed for the level of proximate components. The results are displayed in Table I.

3.1.1. *Moisture Content*

The moisture level of the yacon leaves samples was between 11.74% and 12.37%. The moisture contents were not significantly different ($p > 0.05$) in the leaves harvested at 2nd, 3rd, 4th, and 5th month. The results for the moisture contents acquired in this study were higher compared to those reported by Obeng *et al*. [22], with a range varying between 4.53%–6.79% in selected African leafy vegetables. It was noted that for samples harvested in the 5th month, the moisture levels were slightly less than those harvested in the 2nd, 3rd, and 4th months. This may be an indication that the moisture content decreased with maturity. A comparable observation by Jane Wanjiku *et al*. [23] reported that plant moisture drops as plants age because senescence starts to set in and the amount of fiber content rises. Yacon plant maturity indicates metabolic and physiological changes that maximize the buildup of dry matter, which, as a result, reduces the moisture content of yacon leaves [23]. This can be used as a potential strategy to lower the risk of deterioration during storage, thereby enhancing the shelf life for yacon leaves [23].

3.1.2. *Crude Fat*

Crude fat content in the yacon leaves showed an increasing trend as the level of maturity increases. The samples harvested at the 2nd month showed a significantly lower crude fat content than those of the 5th month as shown

TABLE I: Proximate Composition of Yacon Leaves Harvested at Different Stages of Growth

Stage of growth	Moisture	Crude protein	Crude fat	Crude ash	CHO	Crude fibre	Energy (kcal/100 g)
2^{nd} month	$12.03^{a} \pm 0.20$	$28.45^{a} \pm 1.0$	$3.51^{b} \pm 0.2$	$10.0^{a} \pm 2.08$	$46.01^{b} \pm 2.83$	$10.56^{a} \pm 0.31$	$1378.4^{a} \pm 33.4$
3^{rd} month	$12.37^{a} \pm 0.5$	$26.80^{a} \pm 2.40$	$3.68^{b} \pm 0.36$	$10.83^{a} \pm 1.85$	$49.32^{b} \pm 2.82$	$10.58^{a} \pm 0.82$	$1412.6^{a} \pm 39.6$
4^{th} month	$12.08^{a} \pm 0.02$	$23.20^{b} \pm 2.14$	$3.80^{ab} \pm 0.14$	$11.06^{a} \pm 1.10$	$49.87^{b} \pm 3.06$	$10.75^{a} \pm 0.72$	$1365.8^{a} \pm 21.3$
5^{th} month	$11.74^{a} \pm 0.55$	$16.75^{c} \pm 0.38$	$4.20^{a} \pm 0.23$	$11.99^{a} \pm 0.16$	$56.65^{a} \pm 0.41$	$10.83^{a} \pm 0.46$	$1386.72^{a} \pm 1.20$

Note: Values are means of triplicates ± standard deviation. Means with different letters in a column have a significant difference.

in Table I. However, it had no significant difference ($p > 0.05$) with the results of the 3^{rd} and 4^{th} month of harvest. Similarly, a report by Seleim *et al*. [24] while working on zucchini vegetables showed no significant difference between vegetables harvested at different levels of maturities. However, the crude fat contents of the yacon leaves from this study showed lower levels than those of Zucchini vegetables that ranged between 5.84%–5.47% [24] but higher than that of orange fleshed sweet potatoes at 1.44%–1.74% [25]. The crude fat contents in the yacon leaves as observed in this study were generally lower ranging between 3.51%–4.20%.

The low crude fat content recorded in the yacon leaves probably makes the vegetables suitable for application in weight management and reduction of chronic diseases brought about by high fat content [26]. Fat acts as an energy source in human diet and thus is a major component in food. In taste perception, fat plays a very key part including helping in the absorption of fat-soluble vitamins in the body. Nevertheless, when in excess, fat can be harmful to the body by causing increased cholesterol levels which can result in cardiovascular diseases [26]. Green leafy vegetables are considered as healthy sources of dietary fat as they are either low or with no fat at all [27].

3.1.3. Ash Content

The crude ash contents ranged between 10.0%–11.99%, as shown in Table I. The results displayed no significant difference ($p > 0.05$) in ash contents of the yacon leaves at the different stages of harvest. However, a slight rise was witnessed on the ash content as the leaves matured from 2^{nd} to the 5^{th} month of harvest. The results agree with the study by Nekesa Waluchio and Nairobi [28], who analyzed cleaves' maturity. Crude ash contents were, however, lower than that reported by Omoba *et al.* [25], who found a range of 12%–15% in orange-fleshed sweet potatoes. Obeng *et al.* [22] found a range of 2.48%–17.16% in some African leafy vegetables which is lower than the ash contents of the present study. High ash content is an indication that the leaves contain high mineral concentration, as explained by Nekesa Waluchio and Nairobi [28]. However, the ash content of the yacon leaves in this study was much lower than the ash content of *C. argentea*, which ranged between 15% and 28%, when compared to other green leafy vegetables [26] at different harvesting stages, and lower than the ash content of orange fleshed sweet potato leaves at 15.49% [25].

3.1.4. Crude Protein Content

The leaves harvested in 2^{nd} month of growth had the highest amounts of protein (28.45%) and were not significantly different ($p > 0.05$) from the leaves harvested in the 3^{rd} month (Table I). The leaves harvested on the 5^{th} month had the lowest significant levels of protein content at 16.75% while those harvested on the 4^{th} month had protein content of 23.20%. From the results, there is a significant difference between the leaves harvested at different stages of growth, and the trend depicted here is that protein content decreased as the maturity increased. The decrease in proteins in the yacon leaves as stage of maturity advances could be attributed to the senescence or nutrient redistribution. During senescence, the leaves age, and their cells start to break down, which leads to the degradation of cellular components such as proteins [26]. This trend is held up by the report of Seleim *et al*. [24] who was researching on zucchini vegetables and the report of Nekesa Waluchio and Nairobi [28] researching on cassava leaves collected at different levels of maturities. As plants age, their nutrients are transferred to other parts of the plant for storage, such as the tuber [26]. This could be the same case with protein content in yacon leaves. Whereas the plant ages, the proteins in the leaves are transferred to the tuber. The implication of these results is that yacon leaves can be utilized as a substitute protein source, especially when yacon leaves are harvested when the leaves are young, as depicted in the study.

3.1.5. Crude Fibre

As shown in Table I, crude fibre contents in the yacon leaves were 10.56%, 10.58%, 10.75%, and 10.83% for 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} months harvest, respectively. There was no significant difference ($p > 0.05$) in fibre contents between the different stages of harvest. A minor increase was detected as the yacon leaves aged. According to Chaiareekitwat *et al*. [29], fibre content increases with maturity because as the plant ages; the more it requires strength that is offered by fibre. Dietary fibre is important in regulating bowel movement and also helps in slowing down uptake of cholesterol, as well as preventing coronary diseases [26]. The results of this research had similarities with the findings of Jane Wanjiku *et al*. [23], who found out that crude fibre increased with maturity in cowpeas leaves.

3.1.6. Carbohydrates Content

The carbohydrates in yacon leaves harvested at different maturities ranged from 46.01% to 56.65% (Table I). This implied that carbohydrates increased as the leaves advanced in maturity. The highest carbohydrate concentration was recorded in the 5^{th} month, which can be explained by the presence of well-developed leaves with a higher surface area exposed to sunlight, which translates to high photosynthetic capacity, hence a high buildup of photosynthetic products [4]. This is reinforced by the report of Adegbaju *et al*. [26], who showed that carbohydrate contents increased with increase with maturity after considering three stages of maturity for *C. argentea* leaves, where carbohydrate content was highest in the third stage, which was the post-flowering stage. However, the results of this research do not agree with the finding of Adebiyi and Oluwalana [30] in research on mushrooms, who noted a decrease in carbohydrate content as the mushrooms matured. It was also noted that the energy content of the yacon leaves was highest in the 2^{nd} month because of the high protein, carbohydrates, and fat contents recorded at that stage. The energy content in the study is comparable to the results of Seleim *et al*. [24] after working on Zucchini vegetables, whereby the energy levels in the vegetables increased with maturity. This could also be attributed to the increase in carbohydrates as the vegetable matures, carbohydrate being the major source of energy [24].

3.2. *Total Polyphenolic Compounds*

The total polyphenolic compounds in this study varied between 81 and 439.33 mg GAE/100 g, as shown in Fig. 1. Yacon leaves harvested in 2^{nd} month had the highest amounts of polyphenolic compounds, while 5^{th} month had the lowest. This showed that TPC decreases as the yacon plant matures. These results correspond with that of Nadeem and Zeb [31], who found out that maturity has a negative effect on phenolic composition and antioxidant activity in *Ficus carica L.* leaves, where they components decreased as maturity of the leaves advanced. Similarly, Jiang *et al*. [32] reported similar results in tea leaves where phenolic compound content was highest in younger leaves. However, Saffaryazdi *et al*. [33] reported an opposite observation in purslane leaves, where the leaves had higher phenolic compounds during the flowering stage. The results in this research indicated that TPC was highest in the early developmental stages. This could be because, during the early stages of growth, the plant requires a lot of protective compounds, including antioxidants [34]. During the early growth stages, plants are more metabolically active as they need to generate higher amounts of these essential compounds for their growth. During later stages of growth, the leaves had lower TPC concentration, which can be attributed to the fact that the yacon plant, during maturation, shifts its focus from growth and development to reproduction [34]. Polyphenols include compounds that are secondary metabolites which confer therapeutic effects in plants and animals [35]. These compounds satiate free radicals in plants and animals due to their ability to donate hydrogen and also their antioxidant activity. This reduces oxidative stress during the growth and maturation of plants [31].

3.3. *Antioxidant Activity*

The yacon leaves' antioxidant activity was determined through their DPPH scavenging activity. The results (Table II) revealed that the antioxidant activity of the yacon leaves decreased with maturity in all concentrations of the samples. Thus, the results agree with what Nadeem and Zeb [31] found out in *Ficus carica L.* leaves, where the antioxidant activity decreased as the leaves advanced in maturity. The decrease in antioxidants could be attributed to the decrease in phenolic compounds (Table II). This is because TPC are the greatest contributor of antioxidant activity through their radical scavenging activity [36]. However, this study's findings do not agree with those of Kirigia *et al*. [4] who reported that antioxidant activity in cowpeas increased as the leaves matured. However, at the 5^{th} month of harvest, the antioxidant activity was higher, notable in all concentrations. This showed that antioxidant activity does not solely depend on TPC. Other compounds, such as vitamins, enzymes, or non-phenolic antioxidants, contribute to the antioxidant activity of leaves [36].

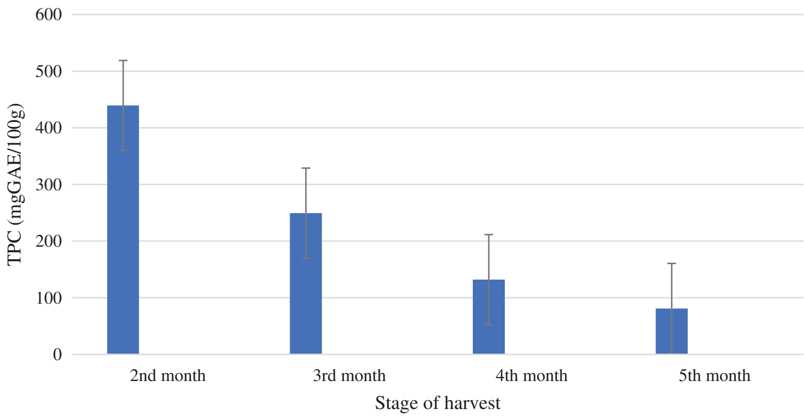

Fig. 1. Total phenolic compounds in yacon leaves harvested at different stages of growth.

TABLE II: Antioxidant Activity in Yacon Leaves Harvested at Different Stages of Growth

Conc (mg/ml) sample	0.375	0.75	1.25	2.5	5
2^{nd} month	$91.70^{ab} \pm 0.21$	$65.62^{a} \pm 5.09$	$43.06^{ab} \pm 1.47$	$26.54^{b} \pm 1.38$	$29.65^{ab} \pm 1.44$
3^{rd} month	$89.25^{b} \pm 1.9$	$50.61^{b} \pm 0.95$	$37.72^{b} \pm 2.24$	$22.78^{b} \pm 2.45$	$22.09^{b} \pm 0.46$
4^{th} month	$76.93^{c} \pm 1.81$	$50.67^{b} \pm 2.11$	$35.14^{b} \pm 1.52$	$27.86^{b} \pm 1.09$	$23.96^{b} \pm 3.56$
5^{th} month	$93.32^{a} \pm 0.69$	$62.42^{a} \pm 7.99$	$50.36^{a} \pm 7.84$	$41.13^{a} \pm 8.78$	$38.25^{a} \pm 10.77$

Note: Values are means of triplicates ± standard deviation. Means with different letters in a column are significantly different.

4. Conclusion

The study investigated the proximate composition, antioxidant activity and total polyphenolic components of yacon leaves harvested at different levels of maturities. It was noted that yacon leaves studied can be a good protein source, especially during the initial stages of growth. Yacon leaves can also be a good supply of carbohydrates and crude fibre, especially when the leaves have matured. The results of this study confirmed that the stage of maturity has an impact on the proximate composition, antioxidant activity, and total polyphenolic compounds of yacon vegetables, and thus, consuming them at various stages is the best way to benefit from the different nutritional components of the yacon leaves. Most components decline as the leaves mature except for carbohydrates and fibre. Thus, yacon leaves could make a significant contribution to the protein content in the diet, and harvesting should be done while the leaves are still at a tender age.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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