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Revealing the Antioxidant, Phenolic and Beta-Carotene Richness of Sweet Potato (*Ipomoea batatas* L) Leaves

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ABSTRACT

The diverse nutrients found in sweet potato leaves, including vitamins, minerals, and antioxidants, offer a range of health benefits. The presence of carotenoids and polyphenols, both powerful health-promoting compounds, highlights their potential contribution to medical science. The main objective of this study was to ascertain essential functionals substances of antioxidant, phenolic compounds, and β-carotene in the leaves of 14 distinct sweet potato lines cultivated in open field. A comprehensive analysis of antioxidant capacity, phenolic content, and β-carotene was conducted using ABTS, Folin-Ciocalteu, and HPLC techniques, respectively. The study showed that the concentrations of total antioxidants, phenols, and β-carotene differed markedly among the leaf materials. Among the lines, SP-13 stands out with the highest concentration of phenols (124.64 mg/g dry weight), while SP-14 comes in at the opposite end with the lowest amount (62.97 mg/g dry weight) under field conditions. In the case of antioxidant content in line SP-3 showed the highest with 3.55 mg/g dry weight, while SP-14 brings up the lowest with 1.88 mg/g dry weight. Line SP-5 showed the most β-carotene (0.51 mg/g dry weight), while SP-11 had the least (0.05 mg/g dry weight). Therefore, it can be concluded that sweet potato leaves are a valuable dietary source of antioxidants, phenolic compounds, and β-carotene which have beneficial health elements.

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Introduction

Sweet potatoes (*Ipomoea batatas*, a delicious, orange-fleshed root vegetable, sprouts from a plant with two seed leaves and belongs to the same family as morning glories. The economic importance of sweet potato has firmly solidified its position as one of the most valuable crops worldwide (Wang et al., 2016). It originated in the tropics of the Americas. This plant belongs to a diverse group of over 1,000 species within the Convolvulaceae family, which encompasses roughly 50 genera. Among the local flora, *I. batatas* are the most prevalent, typically found growing directly in the ground. This root tops the global charts for carbohydrate intake, making it a dietary staple for many people. Generally, the leaves and petioles from the sweet potatoes are discarded back into the field. Research by Xu et al. (2010), indicates that, in some instances, the leaves have been repurposed as a dietary supplement for animals. Research by Islam (2006, 2014) suggests that the consumption of sweet potato leaves as fresh vegetables is common not in certain regions yet. Several nations did not adopt sweet potato leaves as a common vegetable, likely due to their unfamiliarity with their impressive nutritional value. Though Asian countries readily incorporate sweet potato leaves into their dishes as spinach-like vegetables, North America has yet to fully embrace this culinary practice.

The nutritional value of sweet potato leaves has been emphasized in many studies (Xu et al., 2010; Taira et al., 2013; Wang et al., 2016). As a result, scientists and growers have become more interested in exploring their production and various applications. Using the leaves as a source of green vegetables can offer a readily accessible and affordable solution to food insecurity and malnutrition in impoverished nations. The health benefits of sweet potato leaves exceed other leafy vegetables in terms of nutritional value. Studies indicate the leaves contain a higher concentration of beneficial substances compared to other components of the plant, such as petioles, stems, and roots (Lako et al., 2007; Huang et al., 2013; Wang et al., 2016). The leaves have been formally acknowledged as a highly nutritious vegetable by the Asian Vegetable

Research and Development Centre due to its abundance of vital nutrients such as minerals, vitamins, and functional micro-components (Liao et al., 2011; Jang and Koh, 2019). In the study, Mau et al. (2020), discovered that sweet potatoes' vines, particularly those with a length of 40–45 cm, had the potential to serve as a feasible substitute for up to 15% of the flour utilized in baking. Consequently, they can be classified as verdant foliage vegetables, fulfilling the role of versatile and nourishing vegetables.

In various regions across the globe, including Thailand, the Philippines, Senegal, South Africa, Ghana, China, India, and Bangladesh, sweet potato leaves are a popular culinary tradition, prepared and enjoyed as cooked vegetables. Beyond just a side dish, sweet potato leaf tips offer a bounty of culinary delights. The tender tips of sweet potato leaves (about 15 cm from the growing point) offer a superior taste experience for consumers compared to the older leaves, as studies have shown (Xu et al., 2010; Sun et al., 2014). The tender leaf tips can be prepared like other leafy vegetables by cooking or frying them. They also add a fresh touch to salads. This plant demonstrates excellent growth tolerance in moderate pH environments and exhibits a high degree of adaptability to tropical and subtropical climatic conditions. Ranking as the sixth most significant vegetable crop worldwide, sweet potato stands out for its exceptional resilience against diseases, pests, and even the challenges of humid climates, surpassing other popular tropical leafy vegetables.

Research demonstrates that the colours of sweet potatoes, primarily orange, white, yellow, and purple, significantly impact their nutritional content (Kim et al., 2011; de Albuquerque et al., 2019). Characterizing the nutritional makeup of diverse sweet potato leaf tip colours is key to maximizing their dietary value. Carotenoids, with their incredible antioxidant abilities and potential to become vitamin A, are crucial for human health. Over 600 unique chemical structures have been discovered for these pigments. The leaves are a good source of carotenoids, which are also found in many other fruits and vegetables. Despite the potential health benefits of sweet potato leaves, their carotenoid content, particularly compared to other leafy greens, remains largely unexplored. The focus of this research was to comprehensively assess the carotenoid composition of sweet potato leaves, with a particular focus on β-carotene.

Materials and Methods

Growing Sweet Potato Plants

This research was performed using tissue cultures of diverse sweet potato lines obtained from the USDA National Germplasm Centre at the University of Arkansas at Pine Bluff and cultivated in open fields. 14 distinct sweet potato lines, designated from SP-1 to SP-14, were selected for evaluation in this study. The slips (cuttings) from 14 sweet potato accessions were planted in sterilized soil into vinyl pots in the greenhouse. The sizes of the growing pots were 0.49 m long, 0.35 m long, and 0.10 m deep. Pro-mix soil (purchased from HTGSUPPLY Co. in Callery, Pennsylvania) served as the substrate for plant growth. The pots were appropriately tagged to identify each line of sweet potato leaves properly. The greenhouse maintained a controlled temperature range of 21°C to 26°C, along with regulated light levels. Following successful bunch formation, three slips for each line were transplanted to the University of Arkansas at Pine Bluff (UAPB) research field to promote vigorous growth and analyze their antioxidant, polyphenol, and β-carotene content in leaves. Plants in the field were spaced 5 cm apart within rows and 10 cm apart between rows. Each plant was marked with a clear and informative label in the field. An NPK fertilizer with a composition of 10% nitrogen, 10% phosphorus, and 10% potassium was applied at a rate of 250 kg per acre. Additionally, a significant amount of compost fertilizer was put at a rate of 3500 kg per acre. An additional 65 kg/acre of ammonium sulphate was applied after each harvest. Before being examined, the leaves collected from the field were meticulously measured, washed with running tap water, packed in plastic bags with labels, and then quickly frozen at a temperature of -81°C. To achieve optimal preservation, the samples were subjected to a freeze-drying process using the advanced MillRock Technology Freeze Dryer (MD3053) from Kingston, NY. The specimens were pulverized into a fine powder utilizing a Hamilton Beach Coffee Grinder (Model: 80335R, Southern Pines, NC, USA), thereafter weighed and stored in polyethylene bags.

Determination of Total Antioxidants

Preparing the ABTS

Antioxidant content was assessed through the TEAC protocol (Miller et al., 1993), with specific improvisation introduced by Tyrakowska et al. (1999). The ABTS reagent (2,2'-Azino-bis3-ethylbenzothiazoline-6-sulfonic acid) and sodium persulfate were combined in a 100 mL volumetric flask by dissolving 0.36 g and 0.0583 g of each, respectively, in water. Following a 12- to 16 h incubation at room temperature in the dark, the ASYS microplate reader (UVM 340) was employed to measure the absorbance of the solution at 734 nm. To prepare a stock solution of sodium persulfate, 0.164 g of sodium persulfate was dissolved in 100 mL of distilled water, resulting in a concentration of 690 µM. A 100 µM stock solution of Trolox was made by dissolving 0.025 g of Trolox in a 10 mL solution of ethanol/methanol combination, which contained 5% ethanol and 5% methanol by volume. The initial concentration of the stock solution was reduced by a factor of five by adding ethanol/methanol at a ratio of 1:4, resulting in a final volume of 5 mL. The total antioxidant capacity of the samples was quantified using a Trolox standard curve consisting of six concentrations (0, 10, 20, 30, 40, and 50 µL). This was achieved by performing linear regression analysis to determine the free radical scavenging effectiveness.

Sample analysis

Using a precision balance, 0.2 g of the ground material was measured and then transferred to a 100 mL beaker. Subsequently, 20 mL of a 70% acetone solution was added. Ultrasonic treatment was done with the FB120 Sonic Dismembrator from Fisher Scientific (Pittsburg, PA) for 20 min at ambient temperature. Following decantation, the sample was centrifuged in a 25 mL tube at 3000 xg for 10 min at 40 °C using an IEC Centrifuge Model-120 (Fisher Scientific Co., Jiangsu, China). The supernatant was collected and kept in a refrigerator at 4°C.

Total phenolic compound analysis

A modified Folin-Ciocalteu assay was implemented based on Makkar et al. (1993), to assess total phenolics in the extracts utilizing tannic acid standards (0, 20, 40, 60, 80, and 100 µL) for calibration. The initial step involved transferring 50 µL of the phenol-containing plant extract, obtained through the extraction process, to a test tube. The volume was adjusted to 450 µL by diluting with distilled water. Subsequently, the solution was subjected to 250 µL of Folin-Ciocalteau reagent, and then treated with 1250 µL of sodium carbonate solution. The solution was vigorously agitated for 30 min using a vortex mixer, followed by an incubation period of 40 min. The absorbance was measured at a wavelength of 725 nm after an incubation period of 40 min. The total phenols concentration, measured in tannic acid equivalents, was estimated by utilizing a calibration curve derived from known tannic acid concentrations.

Extraction of Carotenoids (β-carotene) from sweet potato leaf extracts

Following Rodriguez-Amaya (1999), with slight modifications, the samples were prepared, extracted, and analyzed to construct a calibration curve to determine their concentration. During extraction, 1.0 g of the dried sweet potato leaves were weighed. The samples were transferred to a mortar and pestle. Then 2.0 g of celite was added to the mortar, and the mixture was ground with a pestle. Acetone (15 mL) was added and swirled to extract the leaves' soluble components. The filter paper was used to filter the solution. Vacuum filtration was also used to filter. After separating the solids, the remaining liquid was carefully decanted into a 100 mL beaker. The 100 mL beaker was placed in a fume hood for 24 h to allow the acetone to evaporate. A 10 mL glass pipette was used to conduct the extraction procedure. Cotton wool was put at the bottom of this pipette and clamped on a stand. Silica gel was added to the height of 6 cm of the pipette and topped up with more cotton wool. Three separate glass bottles were used to keep hexane, hexane: acetone (90:10), and acetone individually. Hexane was added to the column until it started coming out of the column. Hexane (2 mL) was added to the beaker containing the dried sample, which was then dissolved using a pipette. The pigment solution was transferred into the column. To remove the pigment adhered to the wall of the pipette, a small amount of hexane was applied. Following the extraction process, a hexane-acetone solution was employed to separate the carotenoids from the stationary phase. Upon collection of the eluate in a 100 ml beaker, it was meticulously transferred to a 50 mL volumetric flask and subsequently diluted using a 90:10 solution of hexane and acetone. The solution obtained (eluate) was filtered using a 0.22 µm PTFE filter and collected in sample vials. A volume of 10 µL of the solution that had been filtered was subsequently introduced into the chromatograph for analysis.

Analysis of High-Performance Liquid Chromatography (HPLC)

The isolation and quantification of β-carotene were performed using HPLC equipment from Shimadzu Co., located in Columbia, MD, USA. In this investigation, the isolation of β-carotene was facilitated using an analytical YMC C30 column with optimum dimensions (250 mm x 4.6 mm) and particle size (5 µm) at room temperature. The separation was achieved by employing a binary mobile phase system including methanol and dichloromethane as phases A and B, respectively. The 10 µL injection required 26 min to traverse the system at a flow rate of 1 mL/min (Table 1).

Statistical Analysis

Triplicate analyses were performed on each sweet potato sample to increase the reliability of the data. ANOVA was used to analyze the levels of total polyphenols, antioxidants, and β-carotenoids across different sweet potato lines and assess if any significant differences were present. Data analysis was performed using the statistical software tool SPSS (Version 27.0, IBM Corp.). Mean values of sweet potato lines were compared using a significance level of 5%, indicating significant changes. The findings of experiments were presented as the average, accompanied by the standard deviation, to demonstrate the extent of variation.

Results

Total Phenol Content

Sweet potato leaves, abundant in polyphenols and other bioactive compounds, are gaining recognition as a nutritional powerhouse. The total phenols content in 14 lines of sweet potato leaf are summarized in Table 2 and Figure 1. A significant difference in phenol levels was observed among the selected sweet potato lines grown in field conditions. SP-13 showed the highest phenol content, with a value of 124.64 mg/g, while line no SP-14 had the lowest content, with a value of 62.97 mg/g. The total phenol content of the following lines SP-3, SP-1, and SP-2 were 108.99 mg/g, 108.06 mg/g, and 106.99 mg/g, respectively.

Table 1. Parameters for the analysis of β-carotene content using HPLC

Item	Conditions and components
Instrument	HPLC
Column	Polymeric YMC C30
Column Specifications	250 mm X 4.6 mm, 5µm
Column oven temperature	80 ℃
Mobile phase	H_2O isocratic
Flow rate	1.0 mL per minute
Detector	RI
Injection volume	10 µL
Retention time	26.0 min
Total run time	35

Table 2. Exploring the field performance of select sweet potato lines: Quantifying antioxidants, phenols, and β-carotene in leaves.

Accession no.	Functional substance content in dry weight (mg/g)		
	Phenol content	Antioxidant content	β-carotene content
SP-1	108.06 ± 3.21g	3.26 ± 0.34b	0.38 ± 0.01i
SP-2	106.99 ±5.67f, g	3.45 ± 0.26b	0.42 ± 0.02j
SP-3	108.99 ± 3.13g	3.55 ± 0.11b	0.14 ± 0.01j
SP-4	88.89 ± 2.82c, d, e	2.90 ± 0.45a, b	0.34 ± 0.02 h
SP-5	87.69 ± 4.31c, d, e	3.14 ± 0.23b	0.51 ± 0.01k
SP-6	88.89 ± 0.64c, d, e	2.79 ± 0.09a, b	0.08 ± 0.01b
SP-7	97.44 ± 2.77e, f	3.35 ± 0.18b	0.30 ±0.02f
SP-8	79.92 ± 0.58b, c	2.70 ± 0.56a, b	0.19 ± 0.02 d
SP-9	76.64 ± 4.38b	1.90 ± 0.53a	0.11 ± 0.10c
SP-10	85.14 ± 2.35b, c, d	3.40 ± 0.23b	0.37 ± 0.00i
SP-11	91.78±0.53d, e	2.64 ± 0.40a, b	0.06 ± 0.01a
SP-12	93.96±1.05d, e	3.17 ± 0.39b	0.38 ± 0.03i
SP-13	12.46±2.07	3.27 ± 0.438b	0.27 ±0.01e
SP-14	62.97±6.50a	1.88 ± 0.65a	0.32 ± 0.01g

Data obtained from three independent replicates (n = 3) are presented as means ± SD on a dry weight basis. The Tukey test indicates a significant difference ($P < 0.05$) between means in the same column with different superscripts.

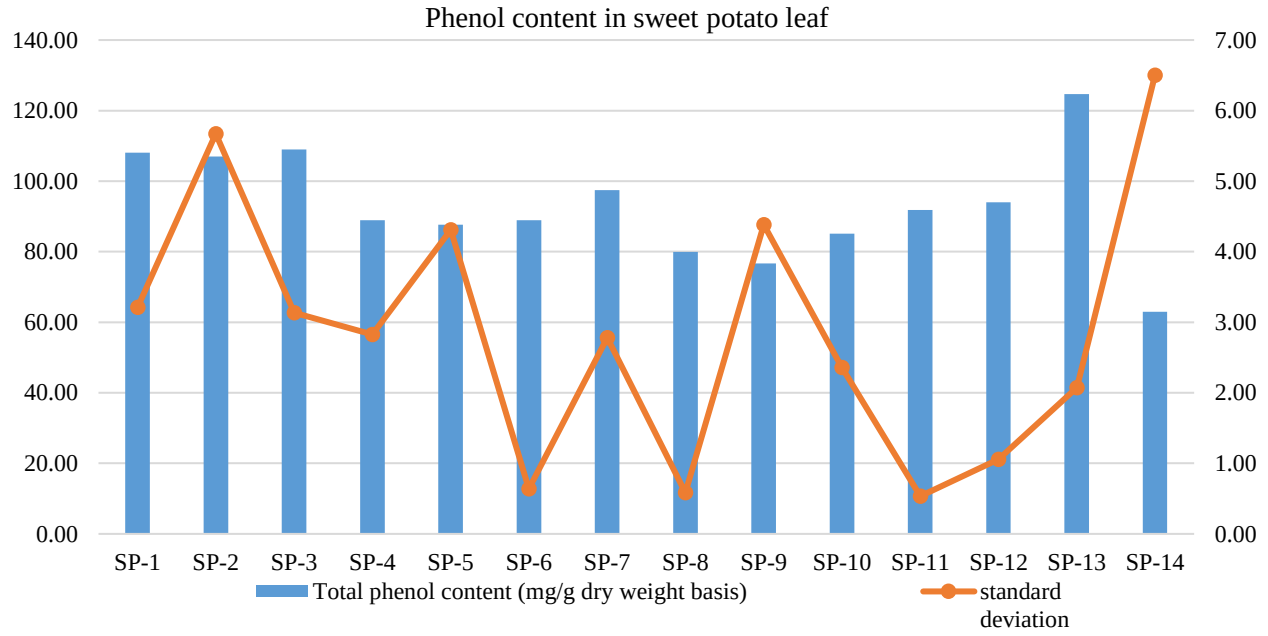

Figure 1. Phenol contents of sweet potato leaf (*Ipomoea batatas*)

Total Antioxidant Content

The antioxidant capacity of 14 lines of sweet potato' leaf was assessed using the ABTS radical scavenging assay. The total antioxidant content (TAC) of field-grown sweet potato leaves exhibited statistically significant ($p < 0.05$) variation, ranging from 1.89 to 3.55 mg/g dry weight across the analysed lines (Table 2 and Figure 2). The antioxidant content of sweet potato leaves varied considerably, with SP-3 reaching a remarkable 3.55 mg/g (dry weight) at the top and SP-14 coming in at the lowest with 1.88 mg/g (dry weight). The total antioxidant contents of the following lines were SP-2 (3.46 mg/g), SP-10 (3.40 mg/g), and SP-13 (3.28 mg/g).

Total β-Carotene Content

The β-carotene content of 14 sweet potato leaf lines grown in the field is summarized in Table 2 and Figure 3. Among the selected sweet potato varieties, SP-5 contained a significantly high amount of total β-carotene (0.51 mg/g dry weight), followed by SP-2 (0.429 mg/g dry weight), SP-3 (0.41 mg/g dry weight), SP-1 (0.38 mg/g dry weight), SP-10 (0.37 mg/g dry weight) and SP-12 (0.37 mg/g dry weight). The concentration of β-carotene was notably lower in the other lines.

Discussion

Research has shown that sweet potatoes can promote overall health, making them a beneficial companion for a healthier lifestyle (Alam et al., 2020c; Luo et al., 2021). The leaves boast a higher concentration of polyphenols than a wide range of popular vegetables, including spinach, kale, amaranth, and cabbage (Kurata et al., 2019; Makori et al., 2020). According to Wang et al. (2016), their leaves exhibit a significantly higher concentration of polyphenols compared to grape seeds, with a 7-9-fold increase observed. In a 2020 study, Cho et al. discovered that leaves are rich in antioxidants called phenolic compounds. These antioxidants showed no harmful effects on cells and may even help boost the immune system, making sweet potato

leaves a potential functional food. Research by Zhang et al. (2015), demonstrated a significant variation in the total phenolic content of Malaysian sweet potato leaves, with values ranging from 3,470 to 5,350 mg/kg on a dry weight basis. Jiang et al. (2019) reported that genetically modified sweet potato cultivars in South Korea had leaves containing an average of 650 to 910 g of phenols per 100 g of fresh weight. A Chinese research team led by Hong et al. (2020), revealed that the phenolic content of sweet potato leaves varied significantly, ranging from 39,400 to 164,400 µg / g when measured on a dry weight basis. Sweet potato leaves boast a diverse range of thirteen phenolic acids, as reported by Luo et al. (2021). The research has demonstrated that sweet potato leaves are a valuable source of polyphenols. Here, SP-1, 2, 3, and 13 have a higher number of phenolic compounds containing sweet potato varieties, and SP-4, 5, 6, 8, and 14 contain less phenol among the types in field conditions. SP-13 is the highest phenol-content variety, and SP-14 showed the lowest phenol-content variety among the field-grown lines.

Antioxidants can also be found in abundance in sweet potato leaves. Research by Luo et al. (2021), reveals that flavonoids and specific phenolic compounds in the leaves have biological properties, most notably antioxidant activity. A study by Nagai et al. (2011), found that various antioxidants in the leaves play a vital role in the natural immune system of the human body. These antioxidants combat harmful reactions, prevent cholesterol from oxidizing, and keep DNA safe from damage in white blood cells. The presence of multiple antioxidant components in sweet potato leaf extracts suggests their possible role in promoting antioxidant activity (Wang et al., 2016). Sweet potato leaf extracts, rich in antioxidants, hold promising potential for developing nutraceutical products with significant health benefits and improved nutritional profiles. Research suggests that sweet potato leaves are a surprisingly potent source of antioxidants, even exceeding the levels found in common leafy vegetables and even their own sweet potato root and potato counterparts (Islam et al., 2014).

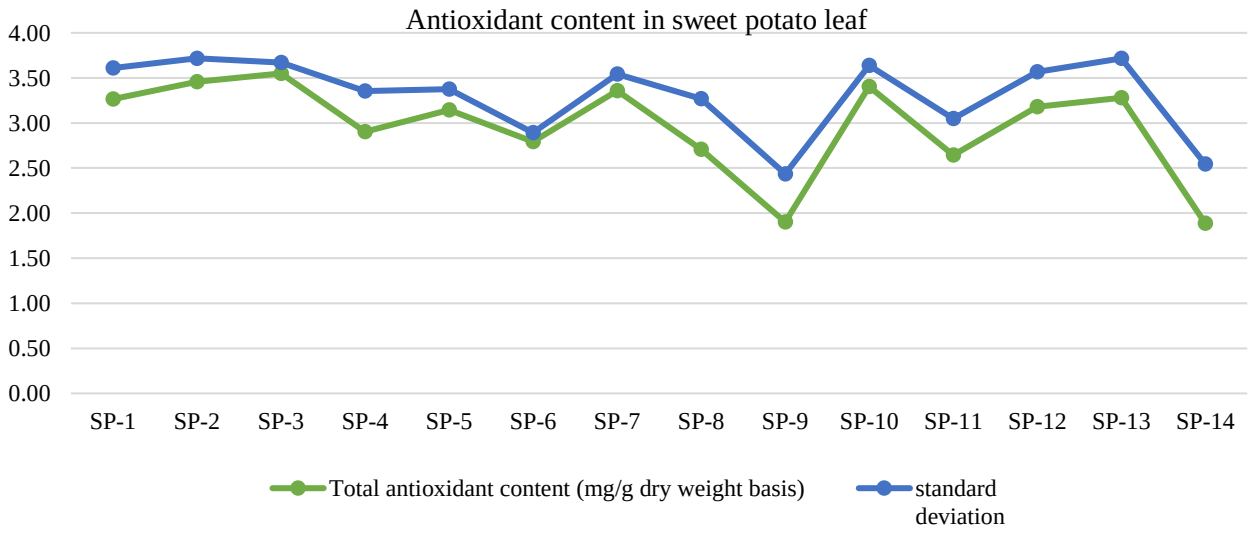

Figure 2. Antioxidant contents of sweet potato leaf (*Ipomoea batatas*)

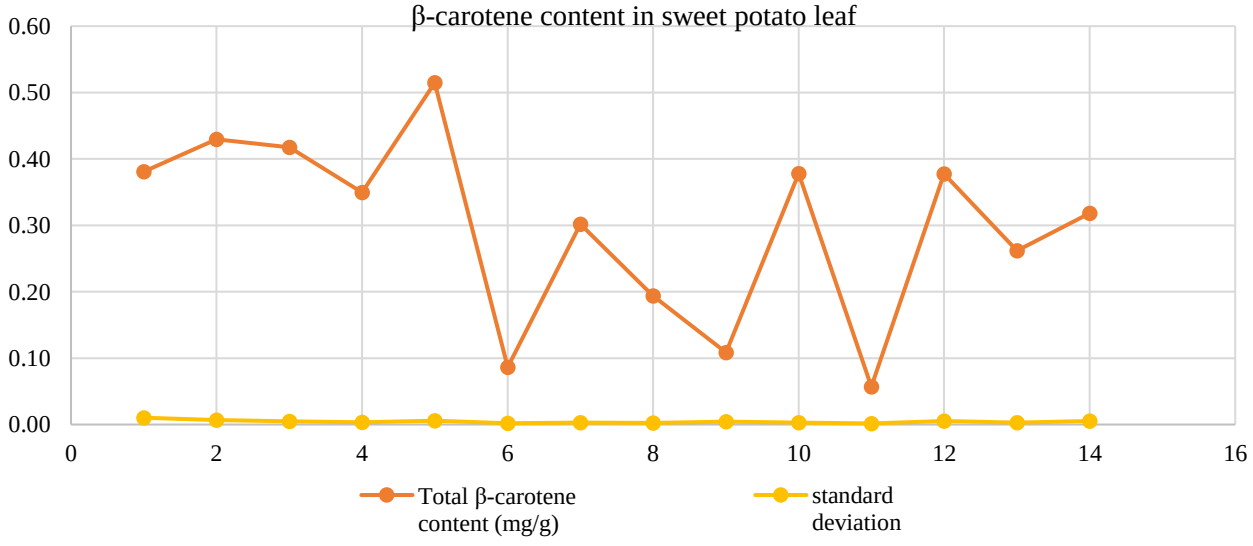

Figure 3. β-carotene contents of sweet potato leaf (*Ipomoea batatas*)

Studies by Makori et al. (2020), and Suarez et al. (2020), have demonstrated that the leaves of various sweet potato cultivars exhibit a range of capacities in terms of neutralizing free radicals. Sweet potato leaves function as a cellular antioxidant immune system, scavenging and neutralizing Reactive Oxygen Species (ROS) that can damage cells (Islam, 2014; Abong et al., 2020, 2021Makori et al., 2020; Suarez et al., 2020). The laboratory evaluates the antioxidant and free radical scavenging properties of the samples using several chemical assays, such as DPPH, ORAC, FRAP, and ABTS. This has been established by Hong et al. (2020), Im et al. (2021), and Sun et al. (2019). In their study, Im et al. (2021), employed the ABTS method to quantify the antioxidant capacity of sweet potato leaves. The results indicated that the antioxidant capacity ranged from 6.10 to 7.66 mg Trolox equivalents per gram when the leaves were dried. In a study conducted by Sun et al. (2014), it was shown that there were notable variations in the antioxidant capacity of leaves among 40 different sweet potato genotypes. This was determined using a photo-chemiluminescent approach. The ascorbic acid content (AAE) in the genotypes varied between 0.08 and 0.82 mg/g of dry weight. Makori et al. (2020), found the leaves exhibited a broad range of antioxidant capacity. A study by Suarez et al. (2020), revealed that leaves are a potent source of antioxidants. Their analysis, employing various methods like DPPH, ABTS, and FRAP, showed impressive antioxidant levels of 7.4 g AAE/100 g DW, 10.6 g AAE/100 g DW, and 0.617 μM TE/100 g DW, respectively. Among the field-grown sweet potato lines we studied, SP-1, 2, 3, 7, 10, and 13 stood out for their high antioxidant content. This suggests that these lines may be particularly beneficial for promoting health. Among field-grown lines, the SP-3 exhibits the highest level of antioxidants, while the SP-14 exhibits the lowest level. The antioxidative capabilities of fruits and vegetables are influenced by a combination of environmental and genetic factors (Islam et al., 2003). The findings of Yooyongwecha et al. (2013), suggest that soil moisture plays a critical role in the growth and development of sweet potatoes. Furthermore, their study identified sunlight, temperature, and moisture as important factors contributing to leaf growth in field settings.

The amount of β-carotene in the leaves of different sweet potato varieties varied significantly. Analysis of sweet potato leaves revealed a wide range of β-carotene content, from 56.97 to 514.69 μg/g (dry weight). SP-5 emerged as the variety with the highest β-carotene content, while SP-11 contained the least. Our analysis of β-carotene content in the leaf sample corroborated the findings of Ishida et al. (2000). Sweet potato leaves, according to their findings, are chock-full of vitamins, including the powerful antioxidant β-carotene. Research by Li et al. (2017), revealed that sweet potato leaves contain significant amounts of β-carotene. Dry weight β-carotene concentrations fluctuated between 352.10 and 520.10 μg/g. The Koganesengan (KS) variety contained significantly more β-carotene (400 μg/g dry weight) than the Beniazuma (BA) variety (273 μg/g dry weight), according to Kandlakunta et al. (2008). Our study identified SP-5, SP-2, SP-3, and SP-10 as promising lines with β-carotene levels comparable to the Koganesengan variety. Conversely, the β-carotene content of the SP-13 and SP-8 closely mirrors that of the Beniazuma variety. Additionally, the β-carotene content of sweet potato leaves and stalks was found to be significantly higher than that of spinach (31.5 μg/g dry weight) and other vegetables (ranging from 0.2 to 17 μg/g dry weight) (Bunea et al., 2008). As green vegetables, sweet potatoes can fulfill the recommended daily requirements of 5000 to 25,000 I.U. of β-carotene due to their high quantities of the compound, often exceeding 8000 I.U./100 g.

Conclusion

The study found that sweet potato leaves cultivated in the field contain a significant number of beneficial compounds, including antioxidants, and β-carotene. The sweet potato leaves and stalks contain multiple functional elements, suggesting their significant potential for use in both fresh and processed culinary applications. Therefore, the consumption and cultivation of sweet potato leaves will yield advantageous outcomes for one's well-being.

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