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DETERMINING THE AMINO ACID, VITAMIN AND ANTI-OXIDANT COMPOUND CONTENT OF DIFFERENT VARIETIES OF KALE

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Abstract

This study was conducted at the agricultural research station of Tikrit University, Saladin, Iraq. The aim was to investigate five varieties of kale plants which had been planted for the first time in this area to evaluate their content of amino acids, vitamins, and antioxidants. The research was designed according to the randomized complete block design with three replicates. The results showed that Nero Di Toscana had significant superiority in most of the characteristics tested. This variety recorded the highest values of essential and nonessential amino acids, vitamins B_1, A, E and K, as well as antioxidants catechin, kaempferol, caffeic acid, ferulic acid and rutin. The highest content of vitamins B_2 and C and the phenol quercetin were found in the Dazzling Blue kale variety. On the other hand, the highest content of vitamin B_3 and sinapic acid were observed in the Dwarf Siberian variety. The Blue Curled Scotch kale exhibited superior performance in vitamin B_6 and antioxidant gallic acid content. It was concluded that the newly introduced kale varieties displayed considerable diversity in biochemical content. This reflects the genetic and environmental variations that affect the synthesis and accumulation of such content with some varieties offering rich nutritional source of

these compounds. These findings provide encouraging grounds for conducting further research in developing and improving these kale varieties.

Keywords: Kale, Varieties, Amino acids, Vitamins, Anti-oxidant compounds.

تشخيص الاحماض الامينية، الفيتامينات والمركبات المضادة للأكسدة في أصناف مختلفة من نبات الكيل

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الخلاصة

اجري البحث في محطة البحوث الزراعية – جامعة تكريت التابعة الى محافظة صلاح الدين – العراق بهدف اختبار خمسة أصناف من نبات الكيل زرعت لأول مرة في هذه البيئة بهدف تقييم محتواها من المركبات الحيوية Biomolecules المتمثلة بالأحماض الامينية والفيتامينات والمركبات المضادة للأكسدة. نفذ البحث وفق تصميم القطاعات العشوائية الكاملة randomized complete block Design وبثلاث مكررات، اظهر الصنف Nero Di Toscana تفوق معنوي على جميع الاصناف قيد البحث في محتوى النبات من الاحماض الامينية المفصولة الاساسية وغير الأساسية، وفيتامينات B1, A, E and K، فضلاً عن مركبات catechin, kaempferol, caffeic acid, ferulic acid and rutin المضادة للأكسدة، وافضل محتوى لفيتاميني B2 and C ومركب quercetin الفينولي حققها الصنف Dazzling Blue Kale، وتحقق أعلى محتوى لفيتامين B3 وللمركب الفينولي sinapic acid عند الصنف Dwarf Siberian Kale، اما الصنف Blue Curled Scotch كانت له الافضلية في محتوى فيتامين B6 ومركب gallic acid المضاد للأكسدة. نستنتج من هذه الدراسة أن زراعة الأصناف المدخلة حديثا للمرة الأولى قد أظهرت تنوعا كبيرا في المحتوى من المركبات الكيميائية الحيوية، مما يعكس التباين الوراثي والبيئي الذي يؤثر في تخليق وتراكم هذه المركبات. وقد أظهرت بعض الأصناف المحتوى الأعلى، مما يشير إلى إمكانية اعتمادها كمصدر غني لهذه المركبات. تُعد هذه النتائج مشجعة للمزيد من الدراسات المستقبلية حول تحسين وتطوير هذه الأصناف.

كلمات مفتاحية: نبات الكيل، الأصناف، الاحماض الامينية، الفيتامينات، مركبات مضادات الاكسدة.

Introduction

Plants rich with bioactive compounds are now widely used as therapeutically-active food (3 and 33). Research informs that risks of cardiovascular and cancer diseases are lowered with proper vegetable diet intakes (2 and 39). There are several plants rich in bioactive ingredients including fatty acids, antioxidants compounds and phenols (13), with the Brassica genus being of specific scientific interest. Studies prove that a diet of cruciferous vegetables reduce the risk of many diseases. Such vegetables are an abundant source of health-promoting substances (23 and 40). Kale is one of the Brassicaceae family species (Brassica oleracea var. acephala) and is an economically significant winter crop that is rated highly as a diet food in Europe, Asia, and the United States (17 and 25). This plant is believed to have been used as a food crop since 2000 BC (29). B. oleracea var. acephala is mainly composed of water (89%), fiber (4%), proteins (3%), fats (1.5%), and is low in carbohydrates (1%) (45). In addition, it is a common source of essential nutrients and satisfies the recommended daily requirements of calcium (25%), vitamin A (100%), and vitamin C (40%) (5). The plant is also a rich source of many chemical compounds such as essential amino acids, vitamins, and minerals (19 and 37).

It is widely used lately as a preventive and therapeutic drug to manage several health conditions such as obesity, inflammation, breast engorgement, constipation, and rheumatism, as well as to prevent cancer and increase anti-cancer activity (7, 11 and 20). Moreover, it is a source of indole-3-carbinol, which helps repair damaged DNA in cells and prevent cancer cells (8). Furthermore, it contains antioxidant, anti-inflammatory and anti-neuropathic compounds including quercetin, kaempferol, and chlorogenic acid (22, 31, 41 and 42). Some bioactive saccharides such as sorbitol, mannitol have also been detected in B. oleracea var. acephala, as well as fructooligosaccharide (FOS) and α-galactooligosaccharide (α-GOS) compounds (16, 30, 32 and 43) which have multiple therapeutic properties. They are used as antibiotic, anti-dietary and constipation fibers as they promote the growth of beneficial bacteria in the colon (34 and 35). Moreover, they help improve mineral absorption, reduce serum phospholipids, cholesterol, and triglycerides (14), and in reducing the risks of intestinal inflammation and colon cancer (24). Despite being a common food worldwide, kale is rarely found in most of the Arab countries, except in some small-scale farms, and is seldom grown locally.

The main objective of this study was to identify the most important biologically active compounds, such as amino acids, vitamins, and antioxidants present in different kale varieties, as well as the potential for growing the plant in Iraq.

Materials and Methods

Location and date of experiment: Field experiments were conducted to investigate different varieties of kale and their genetic variations in the content of amino acids, vitamins as well as antioxidant phenolic compounds. The experiment took place at the agricultural research station of the Department of Horticulture and Landscape, Tikrit University (Longitude: 43° 40' 43.00" E and Latitude: 34° 36' 56.92" N) during fall 2022.

Table 1: Weather data during the experiment period.

Month	Total monthly solar radiation (Mj/m2)	Relative humidity (%)	Min relative humidity (%)	Max relative humidity (%)	Temp. (^{0} C)	Min temp. (^{0} C)	Max temp. (^{0} C)	Precip. (mm)
Oct	12.89	40.70	14.59	66.91	25.87	16.37	34.97	**0**
Nov	9.46	60.00	30.97	85.34	17.36	10.46	24.78	**0.437**
Dec	8.26	70.68	49.44	91.92	13.18	7.67	18.70	**0.69**
Jan	8.50	74.21	47.71	93.26	10.16	5.43	16.19	**1.023**

Source: Agricultural Meteorology Center (agromet.gov.iq).

Experimental design: This experiment was based on the randomized complete block design (RCBD), with one factor and three replicates.

Treatments applied: This was a single factor study involving five varieties of kale. The kale seeds were provided by the Baker Creek Heirloom Seed Company, USA. They were marked and displayed as shown in Table 2.

Table 2: Botanical description of the kale varieties studied.

Origin and year	Kale variety	Description
USA 2021	Dazzling Blue (V_1)	Called blue or purple kale. Its leaves are similar to chard leaves and tend to be blue or purple in color. Their texture is leathery. The blade is broad, with large, visible veins. The variety is frost resistant.
	Dwarf Siberian (V_2)	Also known as the Russian variety. Has a delicious taste and small, soft, patterned leaves. Its leaves and taste are similar to arugula.
	Blue Curled Scotch (V_3)	Characterized by dark green, lobed leaves. Called curly kale due to its wrinkled and rough leaves. Its taste is similar to that of lettuce
	Tronchuda (V_4)	Called Portuguese cabbage because its taste and leaves shape are similar to cabbage. The leaves color is light green. This variety is tolerant to temperatures compared to other varieties.
	Nero Di Toscana (V_5)	In Arabic, this variety is called the dinosaur kale due to the large size of its leaves. It is considered the best kind of kale, and dates back to the 1820s. Distinguished by its beautiful leaves that are dark green and tending to black color, with leathery texture. A single leaf is approximately 1 meter long. Its taste is slightly hot.

Planting arrangements: Preparations such as plowing, leveling, smoothing, and fertilizing of the soil were first done (15), and the soil analyzed to determine its chemical and physical properties (Table 3). Three replicates of five experimental units (2*0.80 m each), were made. The distance between replicates was 1m and 10 seeds were placed alternatively on both sides of the experimental unit. The seeds were sowed 0.3 m apart on October 7, 2022 and a drip irrigation system was utilized to water them together with other agricultural processes as needed.

Table 3: Chemical and physical properties of the soil.

Soil properties	Values	Units
pH	7.6	--
Ec	1.63	**Ds m^{-1}**
Lime	197.4	**g kg^{-1}**
Gypsum	59.6	**g kg^{-1}**
Organic matter	2.1	**%**
Sand	604	**g kg^{-1}**
Silt	233	
Clay	163	
Texture	**Sandy silt**	

Characteristics studied: Samples of the plant leaves were collected on January 12, 2023, for the following purposes:

(a) Amino acid diagnosis: The extraction process was carried out according to the method by (21). An amino acid analyzer instrument was used in compounds estimation based on the chromatographic separation technique according to (9).

(b) Vitamin identification: The compounds were measured using high-performance liquid chromatography based on (18).

(c) Antioxidant phenolic identification: After the extraction and purification processes, 100 µL of the sample was injected into the SYKAMN HPLC system (Germany) equipped with a C18-ODS column (250 × 4.6 mm, 5 µm) (27).

Statistical analysis: All data were collected and measured according to Duncan's multiple range test at p=0.05. Statistical analysis software was used in this function (36).

Results and Discussion

Essential amino acids: The results show that the concentrations of amino acids differed depending on the variety of kale involved (Figure 1). The V_5 variety was significantly superior in all the essential amino acids comprising lysine, methionine, threonine, valine, isoleucine, leucine, phenylalanine, and histidine with values of 20.58, 24.56, 26.55, 24.56, 22.56, 26.88, 25.98, and 24.56 µg g^{-1}, respectively. For the V_4 variety the values for lysine, threonine, isoleucine, leucine, and histidine were 18.59, 20.65, 19.58, 23.65, and 20.33 µg g^{-1}, respectively. V_2 came next recording values of 20.61, 21.65, and 23.05 µg g^{-1} for methionine, valine, and phenylalanine, respectively. The lowest values were found in the V_3 variety.

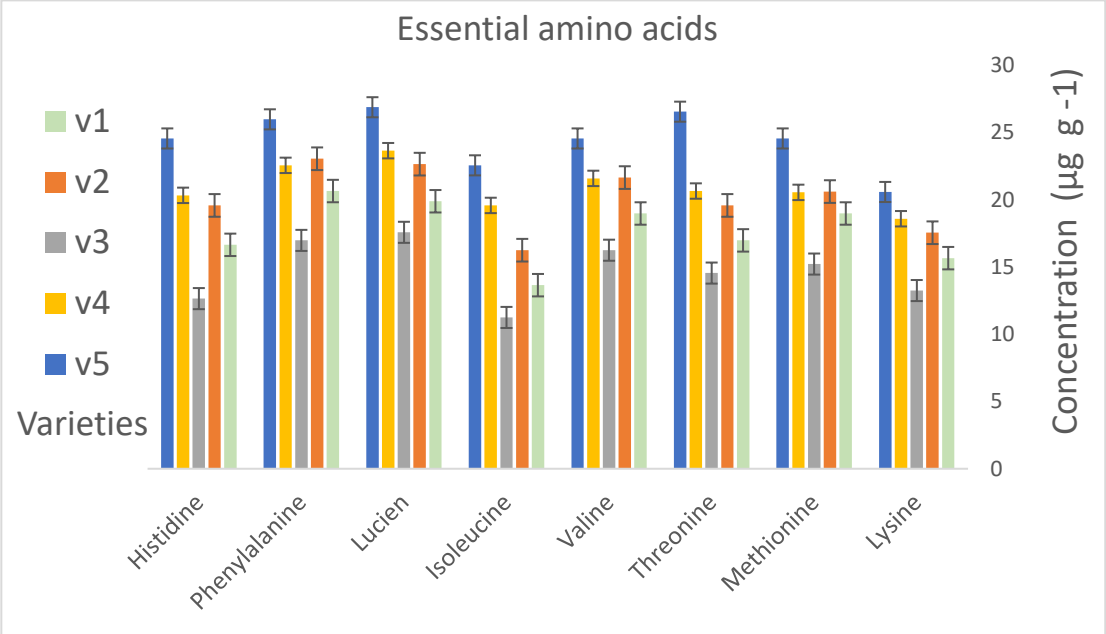

Figure 1: Essential amino acid content in different kale varieties, V_1: Dazzling Blue; V_2: Dwarf Siberian; V_3: Blue Curled Scotch; V_4: Tronchuda; and V_5: Nero Di Toscana.

Nonessential amino acids: There were significant differences in the concentrations of nonessential amino acids in the varieties studied. The V_5 variety had the highest values for arginine, aspartic, serine, and cysteine at 28.99, 29.58, 25.99, and 26.98 µg g^{-1}, respectively. Except for serine, the values of the others were higher by 10.4, 5.7, and 16.3%, respectively in V_2. However, V_2 recorded the highest value for glutamic acid at 25.45 µg g^{-1} while the lowest was for V_3 (Figure 2).

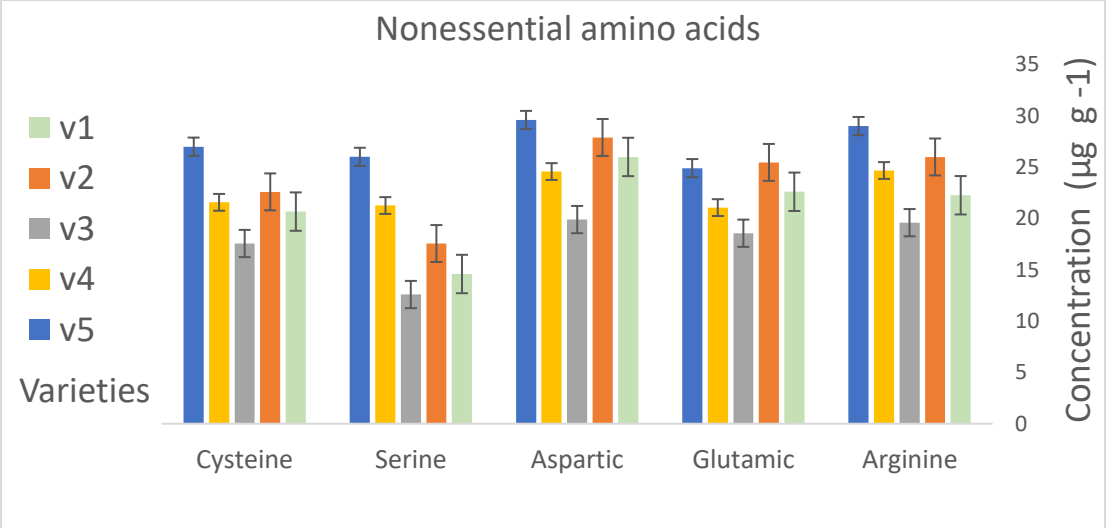

Figure 2: Nonessential amino acid content in different kale varieties, V_1: Dazzling Blue; V_2: Dwarf Siberian; V_3: Blue Curled Scotch; V_4: Tronchuda; and V_5: Nero Di Toscana.

Vitamin content: The results of the analysis show the significant influence of the kale variety on vitamin content. The values for vitamin B_1, A, E, and K were the highest at 0.68 µg g^{-1}, 128.9, 2.1, and 0.32 IU for V_5 (Figure 3-a, e, g, and f). The lowest values were for B_1, A, and E in V_1 (Figure 3-a, e, and g), while K content was the lowest in

V_4 (Figure 3-f). On the other hand, V_1 developed the most content of B_2 (0.66 µg g^{-1}) and C (90.58 IU). Conversely, V_2 contained the lowest B_2 content (0.51 µg g^{-1}) and V_3 the lowest C content (80.25 IU) (Figure 3-b, h). However, as seen in Figure 3-c, the highest value for B_3 at 3.11 µg g^{-1} was in V_2. This variety recorded percentage increases of 3.2% over V_5 which had the second highest value (3.01 µg g^{-1}). The V_1 variety produced the lowest content of this vitamin (2.35 µg g^{-1}). The results in Figure 3-d show the significant superiority of V_3 in B_6 content (1.7 µg g^{-1}) followed by V_5 and V_4 at 1.5 and 1.3 µg g^{-1}, respectively.

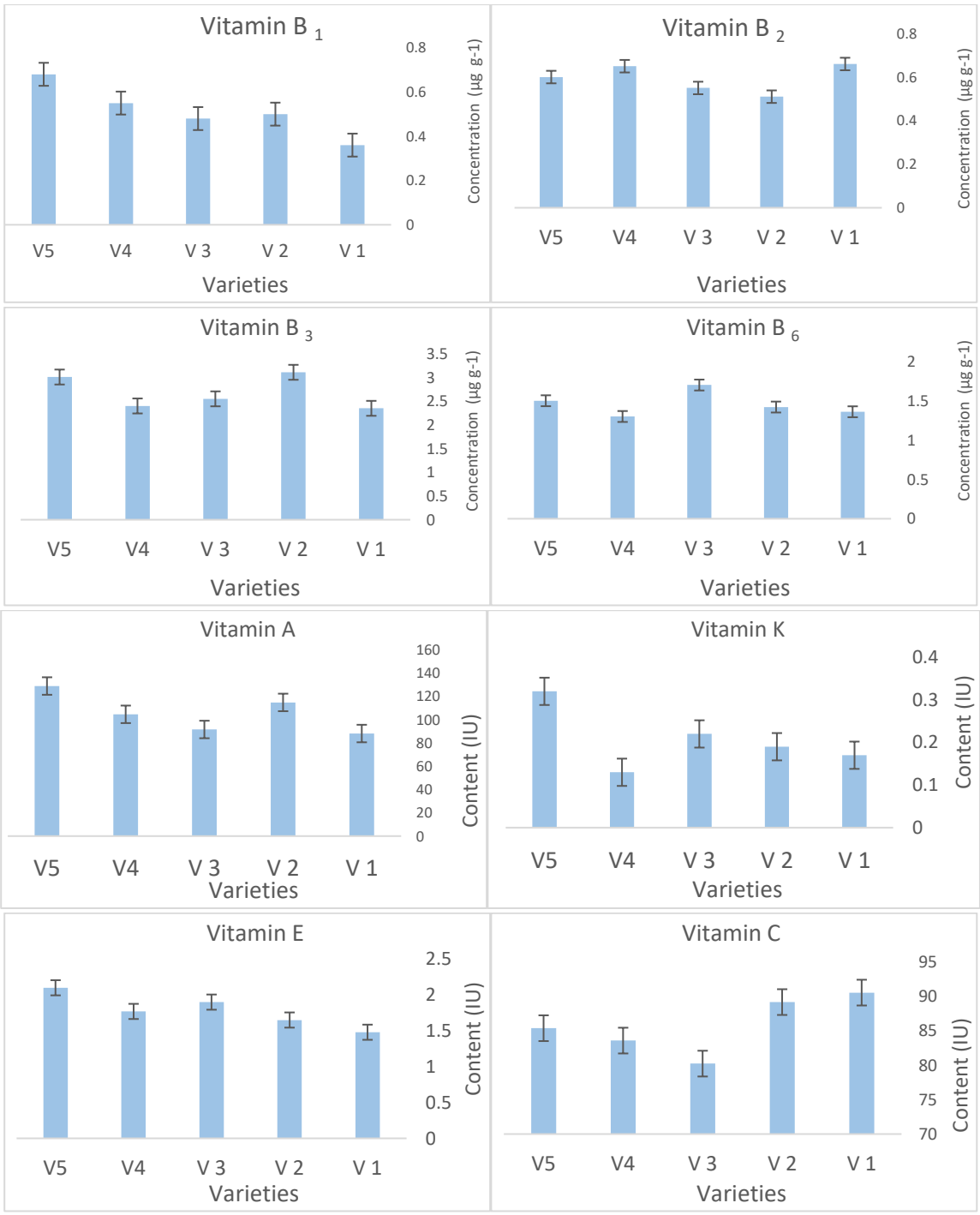

Figure 3: Vitamin content in different kale varieties, V_1: Dazzling Blue; V_2: Dwarf Siberian; V_3: Blue Curled Scotch; V_4: Tronchuda; and V_5: Nero Di Toscana.

Phenolic compounds (antioxidants): Figure 4 shows the significant variations in the phenolic compound content of the kale varieties. The V_5 variety contained the highest values for catechin, kaempferol, caffeic acid, ferulic acid, and rutin at 25.11, 33.08, 22.14, 38.08, and 23.69 mg kg^{-1}, respectively. The lowest values for these compounds (15.47, 25.88, 11.08, 22.88, and 13.58 mg kg^{-1}) were observed in V_1, V_1, V_3, V_1, and V_3, respectively. Quercetin was significantly higher (36.25 mg kg^{-1}) in V_1 followed by 33.58 mg kg^{-1} for V_3 while the lowest value was found in V_2 at 16.08 mg kg^{-1}.

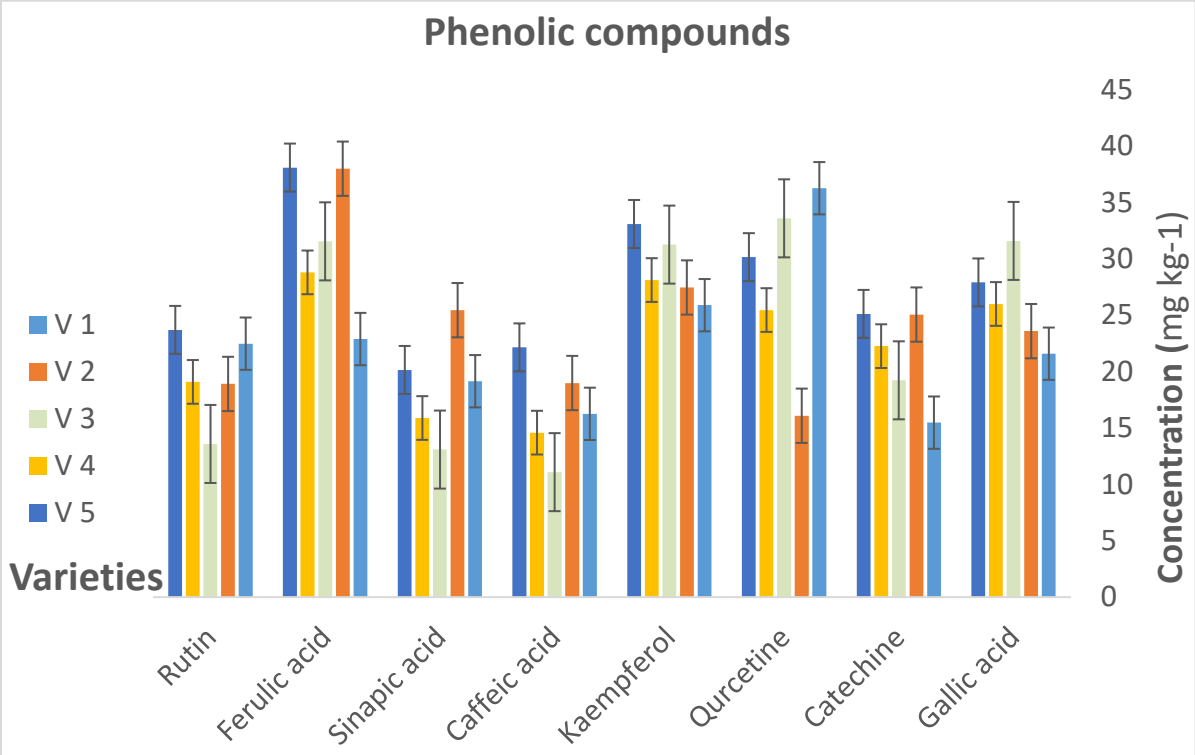

Figure 4: Phenolic compound content in different kale varieties, V_1: Dazzling Blue; V_2: Dwarf Siberian; V_3: Blue Curled Scotch; V_4: Tronchuda; and V_5: Nero Di Toscana.

The differences between compounds (Figures 1, 2, 3 and 4) were due to the genetic variations in the varieties studied. Biosynthesis of active compounds in plants differ based on differences in genetic expression genes. These genes are responsible for the building of those compounds. This was stated by (44) who concluded that chemical compound content varied due to different gene expressions in the kale varieties. (28) confirmed that genes responsible for active compound production had different expressions between the kale varieties. In their study on seven varieties of kale, (6) reported variations in active compound content which can be attributed to their genetic variations and structures. In addition, the extent of gene expression varied.

The variations in vitamin content (Figure 3) may be due to genetic variance between the varieties. It was reported that each genetic structure differs from the other in compound content (26). (38) indicated that concentrations of amino acids differed according to the different varieties. That was attributed to the difference in genetic structure of each of the nine varieties examined. In addition, the researchers noted that food conversion and protein metabolism processes were not similar among those varieties. Further, there was a difference in enzyme activity related to nitrogen and

carbon metabolism, which is necessary for amino acid production. The different vitamin content in Brassicaceae family may be due to variations in the multiple genetic patterns which lead to different expressions of vitamin synthetic genes (10).

The reason for the variation in antioxidant phenolic contents of the different types of kale can be attributed to genetic variations. Plants of the brassica genus differ in their antioxidant content due to variations in their genetic source and structure. Such variations are essential for plant breeding and improvement programs (1). According to Figure 4, the variations in phenol contents of the different varieties may be because of their different genetic structures, endowing superiority for some varieties over others. Therefore, some of these verities were outstanding in specific compounds rather than others (6). This may also have created a difference in metabolic processes allowing the accumulation compounds in the plant. It may also be due to differences in the genetic expression of related genes. In turn, some varieties performed significantly higher than others (4). This agrees with (12) who concluded that variances in antioxidant activity were caused by genetic differences between the five kale varieties studied.

Conclusions

This study showed that the five varieties of kale grown under the environmental conditions of the Salah al-Din region in Iraq varied in their content of biological compounds. The initial evaluation showed different nutritional and medicinal ratings for the varieties involved. The V_5 variety was superior in essential and non-essential amino acid content as well as in vitamins and phenolic compounds. The V_1, V_2, and V_3 varieties had relatively equal values for some of the contents. These varieties can be the choice for agricultural programs aimed at improving the quality of crops grown under similar conditions. The findings reveal the importance of selecting plants based on their nutritional and biological compound content. This helps in meeting agricultural and consumer needs in different regions. This study can be the key towards enhancing research for improving and managing kale cultivation in Iraq. However, further studies are recommended to understand the environmental and genetic factor effects in the concentration of biological compounds. Intensive research on V_5 is highly recommended to benefit from its advantages. Additional varieties of kale and their genetic and biological compounds can be evaluated under different environmental conditions.

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No Supplementary Materials.

Author Contributions:

Author 1: methodology, chemical analysis; Author 2: data analysis; Authors 3 and 4 writing original draft preparation. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

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