

Georgia State University

ScholarWorks @ Georgia State University

Nutrition Masters Projects

Department of Nutrition

Summer 7-31-2023

Evaluation of Nutrient and Phytochemical Content as well as Antioxidant Activity in Hydroponic- and Soil-Grown Kale Extracts

Alivia Setka

Follow this and additional works at: https://scholarworks.gsu.edu/nutrition_mastersprojects

Recommended Citation

Setka, Alivia, "Evaluation of Nutrient and Phytochemical Content as well as Antioxidant Activity in Hydroponic- and Soil-Grown Kale Extracts." , Georgia State University, 2023.
doi: <https://doi.org/10.57709/35894900>

This Project is brought to you for free and open access by the Department of Nutrition at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Nutrition Masters Projects by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.

EVALUATION OF NUTRIENT AND PHYTOCHEMICAL CONTENT AS WELL AS
ANTIOXIDANT ACTIVITY IN HYDROPONIC- AND SOIL-GROWN KALE EXTRACTS

By

ALIVIA MAXINE SETKA

B.S., Georgia State University, 2021

A Master's Project Submitted to the Graduate Committee
in the Department of Nutrition at Georgia State University in Partial Fulfillment of the
Requirements for the Degree

MASTER OF SCIENCE

ATLANTA, GEORGIA

2023

1. Introduction

The availability of arable land is projected to decrease significantly, reaching only one-third of what was available in 1970 by the year 2050 [1]. Traditional farming methods, which demand extensive land and water resources, face considerable challenges due to this reduction in agricultural land [2]. Factors contributing to this decline include global population growth, urbanization, climate change, and poor soil practices, leading to lower crop yields and limiting access to fruit and vegetables [1, 3]. The consequences pose a major global challenge concerning an increase prevalence of food insecurity and chronic disease development [4]. Addressing these complex issues is vital for securing long-term health for individuals, communities, and the environment [5].

As a sustainable alternative to traditional farming methods, hydroponic agriculture emerges as a viable option. Hydroponics is a soilless farming style and involves supplementing the essential nutrients found in soil directly into the water [6]. Commercial hydroponic farmers have been utilizing buildings and shipping containers to grow plants vertically [1]. The indoor environment provides consistency in the growing conditions of the plants with nutrients, pH, humidity, carbon dioxide (CO₂), light, and temperature being controlled, allowing plant growth and development to be maximized [1, 7]. In addition, the indoor, controlled environment allows plants to be produced year-round, regardless of soil quality or land available, and is protective against unwarranted weather such as droughts, hail, and floods [1]. Furthermore, indoor, vertical hydroponic systems are reported to produce higher yields, use less water and land compared to traditional farming, making this a sustainable technique, especially in dry and urban areas [1, 4].

Kale (*Brassica Oleracea*), a vegetable capable of growing easily both in soil and soilless conditions, is a major *Brassicaceae* vegetable consumed worldwide. Kale is strongly associated with reducing the risk of non-communicable diseases due to kale being a rich source of macro- and micronutrients and particularly, secondary metabolites [8-10]. These secondary metabolites, such as polyphenols and glucosinolates, are developed by the plant as a response to stress, serving as a defense mechanism without being essential for the plant's daily functioning [6, 8, 10]. In human health, these phytochemicals play a role in preventing and mitigating oxidative stress due to their antioxidant properties and are associated with the reduction of degenerative diseases, diabetes, cardiovascular diseases, and cancer [8, 10, 11].

To extend the shelf-life of plants, excess water is removed to prevent microbial decay, commonly through dehydration [12, 13]. Various drying methods are available, differing in price, time, and quantity. However, it should be noted that some phytochemicals are sensitive to heat and oxygen and can degrade in their presence [12, 14, 15]. Freeze-drying is considered the most effective method as it does not require heat, but it is time-consuming and expensive [12]. On the other hand, oven-drying methods necessitate higher temperatures but is simple and fast [8].

Due to the novelty of hydroponics in comparison to traditional farming, data on its benefits, quantity, quality, and efficiency remain inconsistent [4]. As such, due to the differences in growing and drying conditions between soil-grown and hydroponic-grown plant foods, it remains unclear whether there are variations in nutrient composition, phytochemical concentrations, and antioxidant activity. Thus, the objective of this study was to compare the polyphenolic and glucosinolate content as well as the antioxidant capacity of hydroponic- versus soil-grown kale and freeze-dried versus oven-dried kale extracts.

2. Methods

2.1 Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescence filters, sodium acetate trihydrate, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid solution (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), hydrochloric acid, iron (III) chloride hexahydrate, sodium persulfate, palladium (II) chloride, sinigrin, Folin-Ciocalteu's Reagent (FCR), sodium carbonate, gallic acid, quercetin, aluminum chloride, and potassium acetate were purchased from Millipore Sigma (Milwaukee, WI, USA). Disodium fluorescein, 2'2'-azobis (2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), acetic acid, and ferric chloride were purchased from Fisher Scientific (Waltham, MA, USA).

2.2 Kale Procurement

Kale (*Brassica oleracea* var. *aceplala*) grown in soil was bought from a local grocery store the morning of the extraction. Kale (Johnny's Seeds, Winslow, ME, USA) was hydroponically grown in an indoor, vertical hydroponic farm with a controlled environment. The humidity and carbon dioxide (CO₂) were kept at 65% and 1200 parts per million (ppm), respectively. To mimic daytime, the light-emitting diode (LED) lights were on for 14 h while temperature was set to 21°C. To mimic nighttime, the LED lights were off for 8 hours, and the temperature was set at 15.5°C (Figure 1).

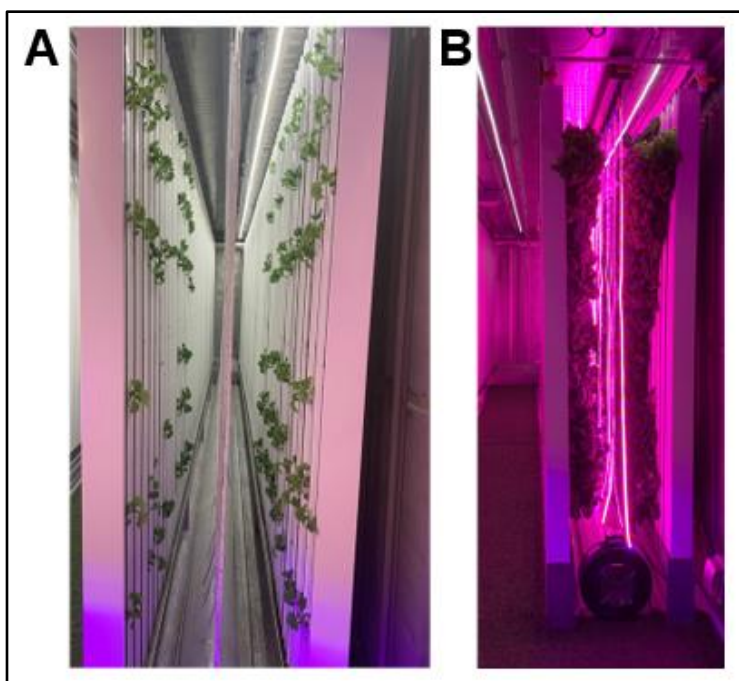


Figure 1. Inside Georgia State University's Hydroponic (A) Young Winterbor Kale in Vertical Towers; (B) LED Lights that Mimic Daytime.

To begin the germination process, two Winterbor kale seeds were inserted into a coco/peat substrate plug (iHort Coco/Peat Grow Plugs, Millstadt, IL, USA). The plugs were secured in a tray then saturated in the seedling trough, which is an ebb and flow system. Once the plugs were completely saturated, the tray was covered with a plastic dome to maintain humidity then placed on a dry rack. After a week, the tray was placed back into the seedling trough. In the seedling trough, seedlings receive a nutrient solution (NS; Table 1) (CleanGrow, FertMax Grow A and B,

Sebastopol, CA, USA) that had an electrical conductivity (EC) of 500 to 600 μS and pH that was maintained at 5.5 to 6.5. After two weeks, the seedlings were transferred to vertical towers, which is a drip hydroponic system (Figure 1). The EC and pH were maintained between 1600 to 1700 μS and 5.5 to 6.5, respectively. The plants grew to full maturity and were harvested the morning of experimentation.

Table 1: Nutrient Comparison of FertMax Grow A (A) and (B) Grow B (B).

A	Nutrient	Percentage	B	Nutrient	Percentage
	Total Nitrogen	4.0%		Total Nitrogen	1.0%
	Soluble Potash	1.0%		Available Phosphate	3.0%
	Calcium	4.2%		Soluble Potash	5.0%
	Magnesium	1.9%		Magnesium	0.89%
	Boron	0.011%		Sulfur	1.3%
	Iron, Cleated	0.06%		Copper, Chelated	0.005%
	Manganese, Cleated	0.013%			
	Molybdenum	0.0007%			
	Zinc, Cleated	0.005%			

2.3 Dehydration of Kale

Soil- and hydroponic-grown kale leaves and stems were washed and cut into small pieces, then either freeze-dried or oven-dried. For the freeze-drying process, samples were initially placed in 50 mL tubes and kept in $-80\text{ }^{\circ}\text{C}$ for 24 h, then freeze-dried (Labconco, Kansas City, MO, USA) under high pressure at $-50\text{ }^{\circ}\text{C}$ for 72 h. For the oven-drying process, kale was spread evenly on a tray and placed into an oven (Thermo-Fisher, Waltham, MA, USA) at 60°C for 24 h. All samples were then pulverized, placed in a clean 50 mL tube, then stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

2.4 Nutrient Analysis

Fresh soil- and hydroponic-grown kale leaves and stems were sent to N.P. Analytical Laboratories (St. Louis, MO, USA) for nutrient analysis.

2.5 Phytochemical Extraction

Polyphenol extractions were performed according to Feresin et al. [16]. Briefly, 10 g of dehydrated kale powder was mixed with 100 mL of 80% aqueous ethanol and placed in an ultrasonic bath (Branson, Danbury, CT, USA) under subdued light with nitrogen gas purging to prevent oxidation. Then, the mixture was vacuum filtered, and the filtrate was collected. The residuals remaining on the filter paper were mixed with 50 mL of 100% ethanol, then vacuum filtered again. The two filtrates were combined and mixed with 50 mL of 80% aqueous ethanol. The solvent was evaporated using a rotary evaporator (Heidolph Instruments, Wood Dale, IL, USA). When the solvent was evaporated, the crude extract was poured into a 50 mL tube with nitrogen injected into the tube for 10 min. Samples were combined with two volumes of chloroform per sample in a separating funnel to form a two-phase mixture. After 1 h, the chloroform layer was discarded, and the aqueous layer was collected. Samples were frozen at $-80\text{ }^{\circ}\text{C}$ for at least 8 h, freeze-dried, then kept at $-20\text{ }^{\circ}\text{C}$ until later analysis.

2.6 Total Phenolic Content (TPC) Assay

To determine TPC, Singleton and Rossi [17] protocol was followed with the modification of using a microplate reader suggested by Ainsworth et al. [18]. FCR was used to determine the TPC of kale extracts. Briefly, samples were prepared in 90% ethanol (1 mg/mL) as were gallic acid standards. Next, 20 μ L of each sample, standard, or 90% ethanol blank was added to a plate followed with 40 μ L of 10% FCR. Then, 140 μ L of 700 mM sodium carbonate was added into each well and incubated for 10 min. The absorbance was read at 765 nm using a microplate reader (BioTEK, Winooski, VT, USA), and the results were expressed as gallic acid equivalents (GAE) (0-800 μ M).

2.7 Total Flavonoid Content (TFC) Assay

TFC was determined using the aluminum chloride colorimetric assay following Chandra et al. protocol [19]. The samples (1 mg/mL) and quercetin standard were prepared in dimethyl sulfoxide (DMSO). Then, 20 μ L of sample or quercetin standard (6.25, 12.5, 25, 50, 80 μ g/mL) were pipetted into a 96-well plate. Next, a mix containing 60 μ L ethanol, 4 μ L of 10% aluminum chloride solution, 4 μ L of 1 M potassium acetate solution, and 112 μ L water per sample were added to each well. The plate was read after 10 min at 415 nm using a microplate reader. Results are expressed as quercetin equivalents (QE).

2.8 Total Glucosinolate Content (TGC) Assay

TGC was determined following the protocol by Ishita et al. [20]. Briefly, 10 μ L of the 1 mg/mL plant extract was mixed with 200 μ L of 2 mM Palladium (II) chloride. After incubating for 30 min at 25 $^{\circ}$ C, the absorbance was read at 425 nm using a microplate reader, and the results were calculated against a standard curve of sinigrin (0, 0.1875, 0.375, 0.75, 1.5, 3, 6 mg/mL).

2.9 Trolox Equivalent Antioxidant Capacity (TEAC) Assay

TEAC was analyzed according to the method of Arts et al. [21]. To perform the assay, 10 μ L of plant sample (1 mg/mL) was added to wells of a clear 96-well plate. Then, 200 μ L of ABTS was added to each well, and the sample was incubated at 30 $^{\circ}$ C for 6 min. The absorbance of the mixture was recorded at 734 nm using a microplate reader, and Trolox was used as the standard (0, 4.7, 9.4, 18.8, 37.5, 75, 150, 300 μ M). Results were expressed as Trolox equivalents (TE).

2.10 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed according to Ring et al. [22]. In brief, 20 μ L of sample (1 mg/mL) was added to each well of a 96-well plate followed by 180 μ L of FRAP reagent, which consisted of 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCL, and 20 mM FeCl_3 at a ratio of 10:1:1. After 10 min, the absorbance was measured at 593 nm using a microplate reader. FRAP values were calculated against the standard curve of iron (II) sulfate heptahydrate ($\text{Fe}^{2+}\text{SO}_4\cdot 7\text{H}_2\text{O}$; 7.8125, 15.625, 31.25, 62.5, 125, 250, 500 μ M).

2.11 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

DPPH was measured according to Manzocco et al. protocol [23]. In short, 50 μ L (1 mg/mL) of sample was mixed with 100 μ L of freshly prepared 0.02M DPPH in wells of a 96-well plate. The

DPPH radical scavenging ability was evaluated after 30 min of reaction time at room temperature. The absorbance was read at 517 nm using a microplate reader and the reaction was calculated against a standard curve of Trolox (2,5,10,25,50,75, 100 μ M). Results are expressed as TE.

2.12 Oxygen Radical Absorbance Capacity (ORAC) Assay

ORAC was determined following a protocol by Ou et al. [24]. Briefly, a 75 mM APPH stock solution and 120 nM of fluorescein solution were prepared separately in phosphate buffered saline (PBS). Then, 5 mM stock solution of Trolox standards prepared with a serial dilution (100-0 μ M). Next, 25 μ L of sample (0.1 mg/mL) and standards were mixed with 150 μ L of fluorescein in a 96-well clear-bottom black plate. The plate incubated for 30 min at 37 °C. Then, 25 μ L of AAPH was added to each well of the plate and read kinetically every minute for 2 h at 37 °C. Results are expressed as TE.

2.13 Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test using GraphPad Prism software (La Jolla, CA). Differences were considered statistically significant when the $P \leq 0.05$. Data are expressed as means \pm standard deviation of the mean (SD).

3. RESULTS

3.1 Nutrition Content Analysis

The nutritional analysis (Table 2) revealed that soil-grown kale exhibited 2.45% and 1.34% more carbohydrates and dietary fiber, respectively, compared to its hydroponic counterpart (Table 1). Moreover, soil-grown kale had a higher concentration of iron, 1.86-fold, and vitamin C, 1.44-fold, when compared to hydroponic kale. On the other hand, hydroponic kale contained more calcium, potassium, and sodium (1.58-, 1.06-, 1.73-fold, respectively) compared to soil-grown kale.

Table 2: Nutrient Comparison Between Soil- and Hydroponic-Grown Kale.

Variable	Soil-grown kale	Hydroponic-grown kale
Calories (kcal/100 g)	42.90	30.80
Moisture (%)	89.30	91.70
Protein (%)	3.36	3.35
Fat (%)	1.02	0.77
Carbohydrates (%)	5.07	2.62
Dietary Fiber (%)	3.64	2.30
Ash (%)	1.19	1.53
Calcium (%)	0.19	0.30
Iron (ppm)	15.20	8.13

Magnesium (ppm)	608	532
Phosphorus (%)	0.05	0.05
Potassium (ppm)	3,860	4,110
Sodium (ppm)	98	170
Folic Acid (ppm)	0.46	0.20
Niacin (ppm)	11.50	11.20
Thiamine (ppm)	<1	<1
Vitamin C (ppm)	316	219

Abbreviations: ppm, parts per million; kcal, kilocalorie; g, grams.

3.2 Polyphenol and Flavonoid Content

Overall, soil-grown kale contained a significantly greater concentration of polyphenols and flavonoids compared to hydroponic-grown kale (Figure 2). Soil-grown oven-dried kale (SO; 324.50 ± 27.14 $\mu\text{mol GAE/L}$) had the highest content of TPC, followed by soil-grown freeze-dried kale (SF; 267.90 ± 17.84 $\mu\text{mol GAE/L}$), hydroponic-grown freeze-dried kale (HF; 131.10 ± 3.74 $\mu\text{mol GAE/L}$), and hydroponic-grown oven-dried kale (HO; 59.80 ± 21.63 $\mu\text{mol GAE/L}$) (Figure 2A). Furthermore, there was a difference between drying methods, with HO having significantly higher TPC than HF ($P = 0.02$), and SO having significantly higher TPC than SF ($P = 0.035$). With respect to flavonoids (Figure 2B), SO (189.80 ± 29.17 $\mu\text{g QE/mL}$) had the highest content compared to all the other kale extracts, followed by SF (134.80 ± 3.24 $\mu\text{g QE/mL}$). SO and SF were significantly different ($P = 0.009$), but there was not a significant difference between HO (21.90 ± 2.58 $\mu\text{g QE/mL}$) and HF (28.30 ± 5.79 $\mu\text{g QE/mL}$).

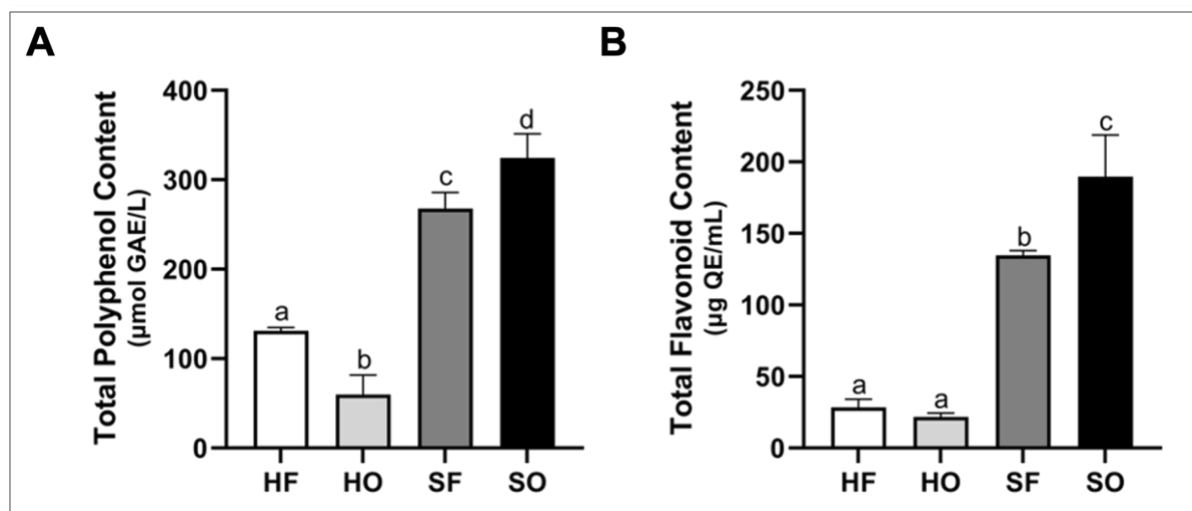


Figure 2. (A) Total Polyphenol Content (TPC) and (B) Total Flavonoid Content (TFC) of Hydroponic- and Soil-grown kale. Data are expressed as mean \pm SD. Values that do not share the same letter are significantly different from each other ($P < 0.05$). HF, hydroponic-grown freeze-dried kale extract; HO, hydroponic-grown oven-dried kale extract; SF, soil-grown

freeze-dried kale extract; SO, soil-grown oven-dried kale extract; GAE, gallic acid equivalents; QE, quercetin equivalents.

3.3 Total Glucosinolate Content

With respect to TGC, there was no significant difference in growing methods. (Figure 3). However, the drying methods used for the phytochemical extraction had a significant effect. HF (1.90 ± 0.40 mg SE/mL) contained significantly higher TGC than HO (1.20 ± 0.14 mg SE/mL; $P = 0.025$), and SO (1.70 ± 0.16 mg SE/mL) had significantly more TGC than SF (1.10 ± 0.06 mg SE/mL; $P = 0.047$).

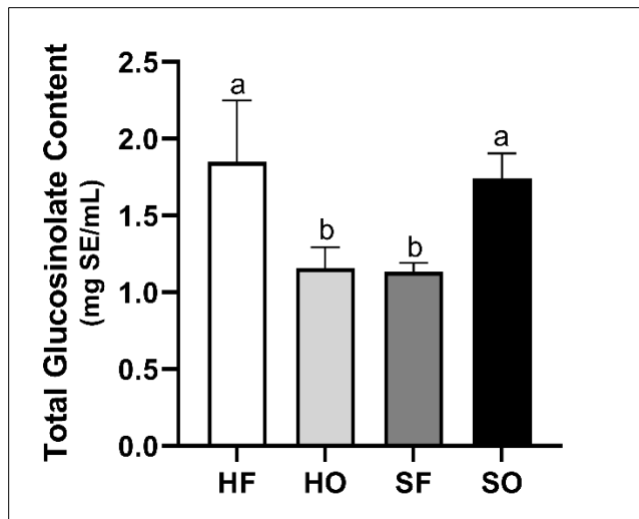


Figure 3. Total Glucosinolate Content (TGC) of Hydroponic- and Soil-grown Kale. Data are expressed as mean \pm SD. Values that do not share the same letter are significantly different from each other ($P < 0.05$). HF, hydroponic-grown freeze-dried kale extract; HO, hydroponic-grown oven-dried kale extract; SF, soil-grown freeze-dried kale extract; SO, soil-grown oven-dried kale extract; SE, sinigrin equivalents.

3.4 Antioxidant Capacity

The antioxidant activity of soil-grown kale as assessed by both TEAC and FRAP was significantly higher than hydroponic-grown kale (Figure 4). With respect to TEAC (Figure 4A), SO (587.70 ± 45.28 $\mu\text{mol TE/L}$) had the highest activity, followed by SF (444.80 ± 10.51 $\mu\text{mol TE/L}$), HO (249.50 ± 45.25 $\mu\text{mol TE/L}$), and HF (220.40 ± 48.66 $\mu\text{mol TE/L}$). There were no significant differences between drying methods used for hydroponic-grown kale. However, a significant difference between drying methods for soil-grown kale was observed, with SO having greater antioxidant activity than SF ($P = 0.011$). With respect to FRAP (Figure 4B), SO (581.60 ± 46.36 $\mu\text{mol Fe}^{2+}\text{SO}_4 \cdot 7\text{H}_2\text{O/L}$) had significantly higher FRAP activity than all the other samples. SO and SF (438.70 ± 15.79 $\mu\text{mol Fe}^{2+}\text{SO}_4 \cdot 7\text{H}_2\text{O/L}$) were significantly different ($P = 0.005$), but HO (124.40 ± 28.13 $\mu\text{mol Fe}^{2+}\text{SO}_4 \cdot 7\text{H}_2\text{O/L}$) and HF (199.50 ± 43.11 $\mu\text{mol Fe}^{2+}\text{SO}_4 \cdot 7\text{H}_2\text{O/L}$) were not.

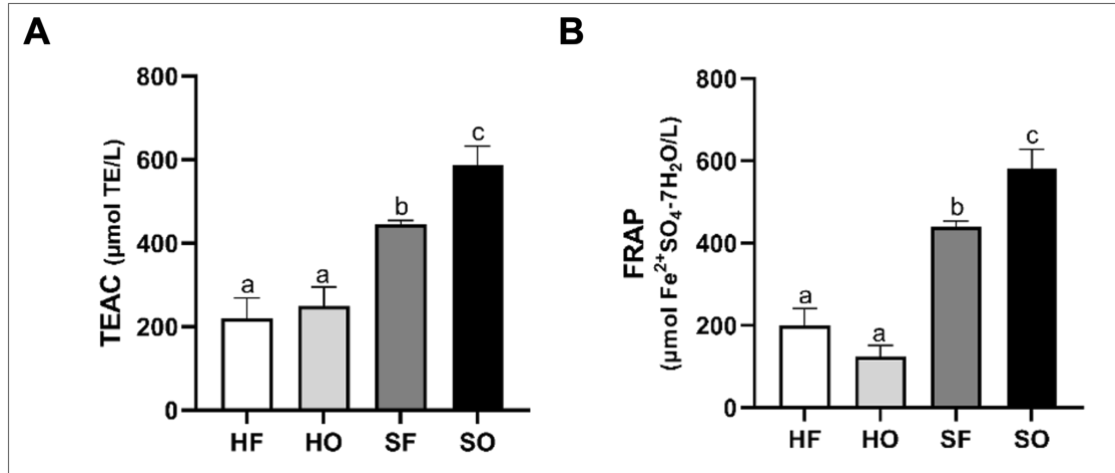


Figure 4. Antioxidant Capacity of Hydroponic- and Soil-grown and Oven- or Freeze-dried Kale Extracts. (A) Trolox equivalent antioxidant assay (TEAC) and (B) Ferric reducing antioxidant power (FRAP) assay. Data are expressed as mean \pm SD. Values that do not share the same letter are significantly different from each other ($P < 0.05$). HF, hydroponic-grown freeze-dried kale extract; HO, hydroponic-grown oven-dried kale extract; SF, soil-grown freeze-dried kale extract; SO, soil-grown oven-dried kale extract; TE, Trolox equivalents.

3.5 Radical Scavenging Capacity

Overall, the radical scavenging capabilities of soil-grown kale were significantly higher than hydroponic kale as assessed by both DPPH and ORAC (Figure 5). With respect to DPPH (Figure 5A), there was no significant difference between SF ($93.69 \pm 6.56 \mu\text{mol TE/L}$) and SO ($108.60 \pm 10.80 \mu\text{mol TE/L}$), but HF ($56.03 \pm 13.09 \mu\text{mol TE/L}$) contained significantly more DPPH activity than HO ($26.79 \pm 10.17 \mu\text{mol TE/L}$; $P = 0.036$). In Figure 5B, SF ($551.80 \pm 3.19 \mu\text{mol TE/L}$) and SO ($548.60 \pm 13.27 \mu\text{mol TE/L}$) had significantly higher ORAC values than HF ($460.20 \pm 15.80 \mu\text{mol TE/L}$) and HO ($385.50 \pm 12.58 \mu\text{mol TE/L}$), respectively ($P < 0.0001$). Further, SF and SO were not significantly different, but the drying methods for the hydroponic-grown kale were, with HF having significantly higher values than HO ($P = 0.0003$).

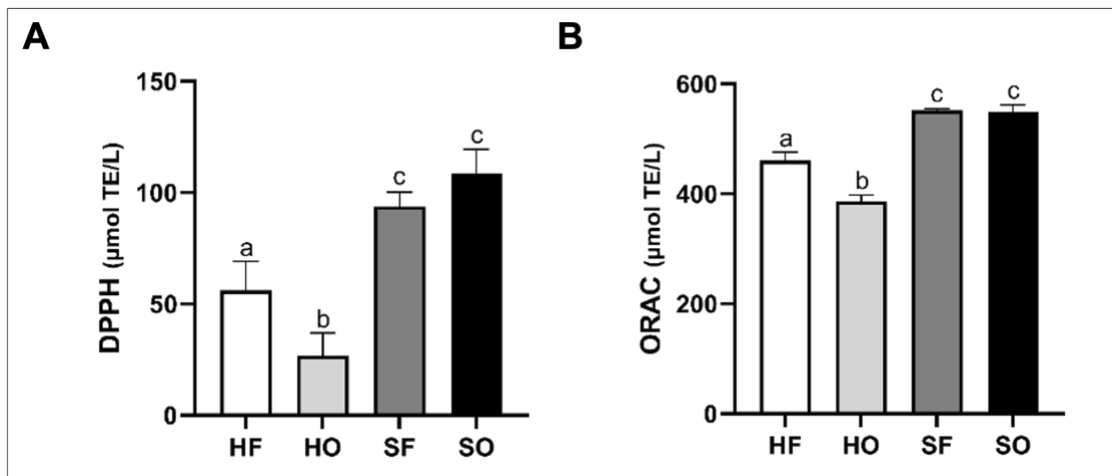


Figure 5. Radical Scavenging Capacity of Hydroponic- and Soil-grown Kale. (A) 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (B) Oxygen reducing antioxidant capacity (ORAC) assay. Data are expressed as mean \pm SD from three independent experiments. Values that do not share the same letter are significantly different from each other ($P < 0.05$). HF, hydroponic-grown freeze-dried kale extract; HO, hydroponic-grown oven-dried kale extract; SF, soil-grown freeze-dried kale extract; SO, soil-grown oven-dried kale extract.

4. Discussion

Traditional agriculture faces many challenges as arable land is decreasing while the population is increasing [1-3]. To reduce the burden of food insecurity and negative health outcomes, hydroponic farming is being explored as a sustainable alternative [4]. To our knowledge this is the first study comparing nutritional and phytochemical differences as well as antioxidant activity between soil-grown kale and hydroponic kale that was oven-dried or freeze-dried. We found that soil-grown kale had superior nutritional quality, polyphenol concentrations as well as antioxidant activity compared to hydroponic-grown kale, with unclear differences with respect to glucosinolate content. These findings are likely due to differences in cultivation, such as temperature, lighting, humidity, and water quality, that can influence the biochemical and nutrient makeup of plants [4, 6]. Furthermore, the nutrient content in plants differ based on many factors, including maturity of the plant and genotype as well as growing and storing conditions [4, 9, 10, 25]. In addition, abiotic stressors are known to influence bioactive compounds in plants, and the effects of the stressors are based on the intensity, frequency, and duration of the stress [26]. Stressors increase reactive oxygen species in plants, and to protect itself from damage, plants develop antioxidants in order to survive [26]. It is worth noting that the hydroponically grown kale in our study was less likely to experience oxidative stress from external stressors compared to the soil-grown kale as 96.5% of agricultural land is affected by abiotic stressors [26]. These variations are a limitation in this study as the plants were not grown in identical growing conditions.

Carbohydrates play a vital role in providing plants with energy, influencing plant growth and development. Sugars can be utilized by the plant immediately or stored to be used when there is a lack of sugar to be absorbed [27]. As such, stress-exposed plants often rely on carbohydrates for survival [4]. In this study, the hydroponic-grown kale potentially does not have as much carbohydrates as soil-grown kale due to lack of stressors. Treftz et al. [4] compared carbohydrate content in soil- and soilless-grown strawberries and raspberries and found that both conventionally grown berries had a higher carbohydrate content than their soilless counterparts, which is similar to the results from our study.

Micronutrients play a crucial role in various plant processes, including photosynthesis and respiration, and the concentration of micronutrients vary during different developmental stages [7, 10]. Among these essential micronutrients, iron is particularly important for photosynthesis, but its absorption availability is low in neutral and alkaline pH [28]. The cleated form of iron used in this study may not have been as stable as the iron available to the soil-grown kale. Therefore, leading to a higher iron content in soil-grown kale. Similarly, vitamin C, is known for its antioxidant properties, which tends to increase in response to oxidative stress [4]. The hydroponically grown kale in this study most likely experienced less stress than the conventionally grown kale. On the other hand, hydroponic-grown kale contained a higher sodium content. A significant challenge in closed hydroponic systems is the accumulation of salts, which occurs when nutrients are not absorbed by the plant and are recirculated, and can result in clogging of nutrient lines as well as reducing the absorption of other ions [29]. However, the kale grown hydroponically also contained a higher calcium and potassium content. This could be due to the higher availability provided to the hydroponic plants by the nutrient solution [29].

Furthermore, our findings indicate that soil-grown kale had higher TPC, TFC, and TGC compared to hydroponic samples. This trend is consistent with other studies that found soil-grown jambu containing more polyphenols and flavonoids, and soil-grown raspberries plants having higher TPC than their hydroponically grown counterparts [2, 4]. Conversely, hydroponically grown strawberries and *Agrimonia Pilosa* contained significantly higher TPC, and hydroponic-grown ginseng roots contained more TPC and TFC than their alternatives [4, 7, 30]. When comparing TFC in *Agrimonia Pilosa*, there was no difference in growing methods [7]. Although there is a lack of data comparing TGC between soil- and hydroponic-grown food, existing literature has demonstrated that glucosinolates tend to increase under extreme light, water, and temperature conditions [10]. However, the glucosinolate content in this study was not conclusive.

Furthermore, soil-grown kale had higher antioxidant activity than hydroponic-grown plants. Similarly, jambu and ginseng roots that were conventionally grown also shown to have higher antioxidant activity than hydroponic-grown counterparts [2, 30]. This can be explained by bioactive compounds, such as phenolic acids, flavonoids, and glucosinolates, being strongly correlated with antioxidant activity [10, 11, 30].

Drying is a commonly employed method to extend the shelf-life of plants by preventing microbial decay [13]. However, the efficiency of drying can vary due to differences in plant structure and phytochemicals properties [15]. We found that SO kale extracts had significantly higher TPC and TFC compared to all other extracts, but HF kale extracts contained higher TPC and TFC than HO. Similar results were found in kale and olive leaves were able to best preserve TPC when oven-dried, and oven-dried ginger was best able to preserve TFC compared to freeze-dried samples [8, 15, 31]. In contract, other studies show that freeze-dried cabbage contained 45% and 53% more in TPC and TFC, respectively, when compared to other drying methods, and freeze-dried ginger contained more TPC than oven-dried ginger [12, 31].

Furthermore, SO and HF had significantly higher TGC than SF and HO, respectively. However, the growing methods were not significantly different from each other. Similar to our results, no significant differences were found in *M. oleifera* that was oven-dried at 40 °C for four days versus freeze-dried for 72 h [14]. Another study found that freeze-dried kale contained more glucosinolates than oven-dried kale [8]. However, our inconclusive results show that the drying effect on bioactive compounds depends on the plant structure and phytochemical properties [15].

Additionally, oven-drying was best able to preserve TEAC and FRAP activity in soil-grown plants, with no significant difference between drying methods in hydroponic-grown plants. On the other hand, freeze-dried hydroponic kale contained significantly more DPPH and ORAC activity than hydroponic-grown oven-dried kale, whereas there was no significant difference in soil-grown plants regarding DPPH and ORAC scavenging activity.

The effectiveness of drying methods may be influenced by the temperature and drying time applied during the process. Although phytochemicals are heat-sensitive, higher temperatures may be able to preserve phytochemicals better due to needing a shorter drying time to remove the same amount of moisture [15]. Freeze-drying may be able to better preserve phytochemicals but necessitates a longer drying time [12].

Overall, conventionally grown plants are more likely to experience stress and are more prone to be attacked by abiotic factors that require an increase in natural defense by phytochemicals, including polyphenols and glucosinolates, and antioxidant activity [4]. However, a strength of this study is that kale was grown in a controlled environment, and different environmental stressors

can be implemented in a controlled hydroponic setting to improve phytochemical content and antioxidant activity in hydroponic-grown produce. In addition, hydroponics has been deemed a better alternative when the amount of arable soil may be a concern [6].

5. Conclusion

In conclusion, soil-grown kale contained significantly higher phytochemicals and antioxidant activity than hydroponic-grown kale. Oven-dried kale extracts contained significantly higher TPC, TFC, TEAC, and FRAP than other extract samples. SO kale had higher TGC than SF kale, whereas HF kale had higher TGC than HO kale. There was not a significant difference in DPPH and ORAC in soil-grown kale regarding drying techniques. However, HF kale contained higher DPPH and ORAC activity than HO kale. The results of this study provide a better understanding on the nutrient and secondary metabolite content as well as the antioxidant capacity of hydroponic- and soil-grown kale. It also provides a foundation for improving the nutritional quality of hydroponic kale. Further research is warranted to explore the interaction of individual environmental factors on plant growth and development and optimization of phytochemicals in hydroponic produce without negatively impacting other components of the plant. Additionally, further research is needed to identify the best extraction and drying methods to better preserve nutrients and phytochemicals.

Acknowledgment

Thank you to Georgia State University's Sustainability Department and Honors College.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

References:

1. Benke, K. and B. Tomkins, *Future food-production systems: vertical farming and controlled-environment agriculture*. Sustainability: Science, Practice and Policy, 2017. **13**(1): p. 13-26.
2. Nascimento, L.E.S., et al., *Phytochemical profile of different anatomical parts of jambu (*Acmella oleracea* (L.) R.K. Jansen): A comparison between hydroponic and conventional cultivation using PCA and cluster analysis*. Food Chem, 2020. **332**: p. 127393.
3. Pozza, L.E. and D.J. Field, *The science of Soil Security and Food Security*. Soil Security, 2020. **1**: p. 100002.
4. Treftz, C. and S.T. Omaye, *Nutrient Analysis of Soil and Soilless Strawberries and Raspberries Grown in a Greenhouse*. Food and Nutrition Sciences, 2015. **Vol.06No.09**: p. 11.
5. McCullum, C., et al., *Evidence-based strategies to build community food security*. J Am Diet Assoc, 2005. **105**(2): p. 278-83.
6. Aires, A., *Hydroponic Production Systems: Impact on Nutritional Status and Bioactive Compounds of Fresh Vegetables*. Vegetables: Importance of Quality Vegetables to Human Health, 2018: p. 55.

7. Wei, H., et al., *Effect of Different Cultivation Systems on the Accumulation of Nutrients and Phytochemicals in Ligularia fischeri*. Horticultural Plant Journal, 2018. **4**(1): p. 24-29.
8. Major, N., et al., *The Effect of Different Extraction Protocols on Brassica oleracea var. acephala Antioxidant Activity, Bioactive Compounds, and Sugar Profile*. Plants (Basel), 2020. **9**(12).
9. Thavarajah, D., et al., *Mineral micronutrient and prebiotic carbohydrate profiles of USA-grown kale (Brassica oleracea L. var. acephala)*. Journal of Food Composition and Analysis, 2016. **52**: p. 9-15.
10. Björkman, M., et al., *Phytochemicals of Brassicaceae in plant protection and human health--influences of climate, environment and agronomic practice*. Phytochemistry, 2011. **72**(7): p. 538-56.
11. Kapusta-Duch, J., et al., *The beneficial effects of Brassica vegetables on human health*. Roczniki Państwowego Zakładu Higieny, 2012. **63**(4): p. 389-95.
12. Xu, Y., et al., *A comparative evaluation of nutritional properties, antioxidant capacity and physical characteristics of cabbage (Brassica oleracea var. Capitata var L.) subjected to different drying methods*. Food Chemistry, 2020. **309**: p. 124935.
13. Managa, M.G., Y. Sultanbawa, and D. Sivakumar, *Effects of Different Drying Methods on Untargeted Phenolic Metabolites, and Antioxidant Activity in Chinese Cabbage (Brassica rapa L. subsp. chinensis) and Nightshade (Solanum retroflexum Dun.)*. Molecules, 2020. **25**(6).
14. Tetteh, O.N.A., et al., *Effects of harvest techniques and drying methods on the stability of glucosinolates in Moringa oleifera leaves during post-harvest*. Scientia Horticulturae, 2019. **246**: p. 998-1004.
15. Ahmad-Qasem, M.H., et al., *Influence of freezing and dehydration of olive leaves (var. Serrana) on extract composition and antioxidant potential*. Food Research International, 2013. **50**(1): p. 189-196.
16. Feresin, R.G., et al., *Extraction and Purification of Polyphenols from Freeze-dried Berry Powder for the Treatment of Vascular Smooth Muscle Cells In Vitro*. J Vis Exp, 2017(125).
17. Singleton, V.L. and J.A. Rossi, *Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents*. American Journal of Enology and Viticulture, 1965. **16**(3): p. 144-158.
18. Ainsworth, E.A. and K.M. Gillespie, *Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent*. Nat Protoc, 2007. **2**(4): p. 875-7.
19. Chandra, S., et al., *Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study*. Evid Based Complement Alternat Med, 2014. **2014**: p. 253875.
20. Ishida, M., et al., *Development of a simple and rapid extraction method of glucosinolates from radish roots*. Breeding Science, 2011. **61**(2): p. 208-211.
21. Arts, M.J.T.J., et al., *Antioxidant capacity of reaction products limits the applicability of the Trolox Equivalent Antioxidant Capacity (TEAC) assay*. Food and Chemical Toxicology, 2004. **42**(1): p. 45-49.
22. Ring, J. and M. Chaung, *The FRAP assay: Allowing students to assess the anti-oxidizing ability of green tea and more*. Journal of Student Science and Technology, 2015. **8**.
23. Manzocco, L., M. Anese, and M.C. Nicoli, *Antioxidant Properties of Tea Extracts as Affected by Processing*. LWT - Food Science and Technology, 1998. **31**(7): p. 694-698.
24. Ou, B., et al., *Determination of total antioxidant capacity by oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescence probe: First Action 2012.23*. J AOAC Int, 2013. **96**(6): p. 1372-6.

25. Korus, A., *Level of Vitamin C, Polyphenols, and Antioxidant and Enzymatic Activity in Three Varieties of Kale (Brassica Oleracea L. Var. Acephala) at Different Stages of Maturity*. International Journal of Food Properties, 2011. **14**(5): p. 1069-1080.
26. Apostolova, E.L., *Molecular Mechanisms of Plant Defense against Abiotic Stress*. International Journal of Molecular Sciences, 2023. **24**(12): p. 10339.
27. Trouvelot, S., et al., *Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays*. Front Plant Sci, 2014. **5**: p. 592.
28. Montejano-Ramírez, V. and E. Valencia-Cantero, *Cross-Talk between Iron Deficiency Response and Defense Establishment in Plants*. Int J Mol Sci, 2023. **24**(7).
29. Maucieri, C., et al., *Hydroponic Technologies*, in *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future*, S. Goddek, et al., Editors. 2019, Springer International Publishing: Cham. p. 77-110.
30. Hwang, J.E., et al., *Comparative study on anti-oxidative and anti-inflammatory properties of hydroponic ginseng and soil-cultured ginseng*. Food Sci Biotechnol, 2019. **28**(1): p. 215-224.
31. Mustafa, I., et al., *Comparison of Phytochemicals, Antioxidant and Anti-Inflammatory Properties of Sun-, Oven- and Freeze-Dried Ginger Extracts*. Foods, 2019. **8**(10).