



Biofortification of kale with vitamin B₁₂ and iodine for vegans using a vertical farming system

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ABSTRACT

Vitamin B₁₂ and iodine deficiencies are significant public health concerns and exhibit greater severity among vegans and vegetarians. This study aimed to evaluate the effects of the multi-biofortification of kale with vitamin B₁₂ and iodine in a hydroponic vertical farming system. During hydroponic cultivation, kale was treated with cyanocobalamin (CN) and KI for a week before harvest. Combination treatment with CN and KI (5 μmol/L each; CN5+KI5) enhanced accumulation efficiency compared with individual treatments, and the consumption of 67.9 g of multi-biofortified kale met the recommended dietary allowances of both vitamin B₁₂ and iodine for adults. Moreover, CN5+KI5 treatment promoted the synthesis of specific secondary metabolites in kale, such as p-coumaric acid and 3,5-dicaffeoylquinic acid. Notably, CN and KI biofortification did not negatively affect kale's growth, visual quality, or sensory properties. Our findings demonstrate that multi-biofortification of kale with CN and KI is a promising strategy for addressing nutritional deficiencies in vegans.

1. Introduction

Micronutrient deficiencies constitute a pervasive public health concern, affecting human health globally; and during the COVID-19 pandemic, the significance of optimal micronutrient intake was underscored (Bhardwaj et al., 2022; World Health Organization, 2023). Vitamins and minerals are essential micronutrients that the human body cannot synthesize; therefore, they must be obtained via dietary means. Deficiencies in these nutrients can result in significant health complications, including impaired immunity, neurodevelopmental disorders, and an elevated risk of chronic illness (Avnee et al., 2023; Godswill et al., 2020).

In recent times, vegan and vegetarian diets have increasingly been adopted. This phenomenon is attributable to a confluence of factors, driven by the impact of climate change, growing environmental concerns, and heightened societal interest in animal welfare and health (Oh et al., 2021). This dietary transition has led to increased concerns regarding plant-based diets with a low bioavailability of essential minerals and micronutrients, such as iron, zinc, vitamin B₁₂, and essential fatty acids (Oh et al., 2021; Park and Kim, 2022). Vitamin B₁₂, in particular, has been highlighted as the most prevalently deficient micronutrient in vegan and vegetarian diets, since most natural sources

of this micronutrient are animal-based rather than plant-based foods (Storz et al., 2024). Notably, certain studies have revealed that vitamin B₁₂ deficiency is not limited to vegans and vegetarian populations (Allen, 2009; Antony, 2003; Pawlak et al., 2013a, 2013b), suggesting that its deficiency is a broader public health concern. Iodine deficiency is also a global public health issue has escalated with the rising number of individuals adopting vegan and vegetarian diets (Krajčovičová-Kudláčková et al., 2003). It can lead to various health problems, including goiter, hypothyroidism, infertility, stunted growth, and intellectual disabilities (Zimmermann, 2009). Since the 2014 Scientific Advisory Committee on Nutrition report regarding the risk of iodine deficiency among vegans and vegetarians, numerous subsequent studies have corroborated the poor iodine status of these groups (Eveleigh et al., 2023).

Considering these circumstances, biofortification represents a sustainable alternative for addressing micronutrient deficiencies. This strategy has recently gained prominence owing to the growing prevalence of food crises resulting from environmental degradation (Bhardwaj et al., 2022; Owino et al., 2022). Biofortification is a cost-effective and sustainable means of improving public health by addressing nutrient deficiencies at the population level while maintaining crop yields and providing higher-quality food (Avnee et al.,

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2023). Vertical farming systems utilize information and communication technologies to support the production of high-quality, standardized crops under precisely controlled cultivation conditions (Van Gerrewey et al., 2021). Vertical farming primarily relies on hydroponic technology and the ability to recirculate nutrient solutions (Van Gerrewey et al., 2021), rendering it an effective approach to biofortification via nutrient solution management. Numerous studies have demonstrated the efficacy of hydroponic systems in fortifying crops with minerals and trace elements, including iodine, selenium, and iron; they have also proposed hydroponics as a novel approach to specialized crop production (Bito et al., 2013; Oh et al., 2021; Puccinelli et al., 2021).

Despite the continued emphasis on the importance of vitamin B₁₂ and iodine intake, research on multi-nutrient biofortification involving these nutrients remains limited. To date, no studies have examined the effects of combined biofortification with vitamin B₁₂ and iodine on crops. This research gap is attributable to several factors. First, vitamin B₁₂ and iodine possess distinct metabolic pathways, physiological functions, and chemical properties; thus, their fortification has been explored in discrete research topics. Second, research into biofortification has typically concentrated on fortification with individual nutrients, rendering studies on multi-nutrient biofortification challenging in terms of both complexity and cost (Avnee et al., 2023; Van Der Straeten et al., 2020). Third, the vitamin B₁₂ content of edible plants is typically extremely low, and its stability and bioavailability vary, limiting the technical feasibility of its effective uptake (Oh et al., 2021). The recent advent of sophisticated agricultural and hydroponic technologies has paved the way for new avenues of research, although these remain in their nascent stages.

Kale (*Brassica oleracea* L. var. *acephala*) reportedly possesses multiple health benefits, including the mitigation of liver disease, obesity, and diabetes, owing to its relatively high levels of calcium; folate; riboflavin; and vitamins C, K, and A, as well as its rich phytochemical content, compared with other cruciferous vegetables (Samec et al., 2019). Furthermore, kale is a valid model plant owing to its ease of cultivation and strong resilience to adverse climatic conditions; therefore, previous studies have adopted kale for biofortification with certain nutrients (Ju et al., 2024; Krzemińska et al., 2024; Son et al., 2021).

The present study aimed to evaluate the effects of combined treatment with vitamin B₁₂ and iodine on kale's nutritional quality, antioxidant capacity, secondary metabolite content, and sensory traits. For controlled production, a vertical farm appliance employing a hydroponic cultivation system was utilized. Ultimately, we sought to develop a specialized crop for populations struggling with vitamin B₁₂ and iodine deficiency.

2. Materials and methods

2.1. Chemicals and reagents

The following chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): cyanocobalamin (CN) standard, sodium acetate, pepsin, trifluoroacetic acid, potassium or sodium cyanide, and α -amylase. Glucosinolate standards were procured from Extrasynthese (Genay, France). Acetonitrile (high-performance liquid chromatography [HPLC] grade) was obtained from Avantor (Radnor, PA, USA), while water (HPLC grade) was sourced from Duksan (Seoul, Republic of Korea). Formic acid was acquired from Daejung (Seoul, Republic of Korea).

2.2. Plant materials and growth conditions

The hydroponic cultivation of kale was conducted using commercial kale seeds (Manchoo collard, a cultivar of *Brassica oleracea* L. var. *acephala* [DC.] Alef, Asia Seed Co., Seoul, Korea). Three seeds were sown in rock wool medium (W × L × H, 25 × 25 × 40 mm; Grodan Co., Roermond, Netherlands), and after 5 days, thinning was performed to retain

a single seedling per medium cube. Harvesting was performed on day 28, with reference to the harvest period recommended by the home-use vertical farming device supplier. Kale was cultivated using a home-use vertical farming device equipped with a hydroponic system (Tiium; LG Electronics, Seoul, Korea). Growth conditions were controlled using the Tiium program, with light intensity and photoperiod set to level 5, maintaining illumination from 8:00 to 22:00 (14:10 h light/dark cycle). The actual measured light intensity was 8540 ± 320 lx. The cultivation temperature was set at 26 °C during the day and 18 °C at night. Fig. 1 illustrates the flow diagram of kale cultivation.

2.3. Plant treatments

A basic nutrient medium was purchased from a home-use vertical farming device manufacturer (LG Electronics, Korea). The nutrient solution was prepared by mixing a concentrated nutrient medium (20 mL) with 3 L of water and replenished in the water tank of the vertical farm, as required. The nutrient solution contained macronutrients and micronutrients, including K (168.87 ± 0.52 ppm), N (138.5 ± 0.50 ppm), P (53.61 ± 0.12 ppm), S (33.38 ± 0.02 ppm), Mg (19.97 ± 0.10 ppm), and Ca (85.88 ± 0.26 ppm). The pH was adjusted to 6.4, and the electrical conductivity (EC) was 1.34 mS/cm. CN is the most stable form among the diverse chemical forms of vitamin B₁₂, and it is chemically synthesized by reacting cyanide with natural cobalamin (Oh et al., 2021). Therefore, CN is widely used in food fortification, biofortification, nutritional supplements, and pharmaceuticals (Nakos et al., 2017; Rizzo et al., 2016) and was consequently adopted in this study. Potassium iodide (KI) is a safe supplement used to support human iodine intake, with iodized salt being a representative food form of KI (Council and Academies, 2004; Todd and Dunn, 1998). Although various forms of iodine have been used in biofortification studies, KI was selected in this experiment owing to its suitability for direct human consumption. Five treatment nutrient solutions were prepared by mixing the base nutrient solution with each of the following: CN 1 μ mol/L (CN1; pH 6.2, EC 1.37), CN 5 μ mol/L (CN5; pH 6.4, EC 1.45), KI 5 μ mol/L (KI5; pH 6.4, EC 1.46), CN 1 μ mol/L + KI 5 μ mol/L (CN1+KI5; pH 6.2, EC 1.41), and CN 5 μ mol/L + KI 5 μ mol/L (CN5+KI5; pH 6.3, EC 1.45). The treatment was applied for 7 days starting from day 21 of cultivation, after which the plants were harvested. The CN (Wellgreen Technology Co., Yanta District, Xi'an, China) and KI (Potassium Iodide Powder, United States Pharmacopeia grade; Prescribed for Life Co., Fredericksburg, TX, USA) used in the nutrient solutions were both food-grade reagents.

2.4. Plant growth measurements

Plant growth parameters were measured weekly to compare growth status among the treatment groups. Leaf length, leaf width, and shoot height were measured using a ruler, while the number of leaves was determined manually. The fresh and dry weights of the epigeal parts were measured using a digital scale (HS224S; Hansung Instrument Co., Gwangmyeong, Korea) after harvesting, with dry weights determined following freeze-drying.

2.5. CN content

The CN content of the kale samples was determined using the method described by Nakos et al. (2017), with certain modifications. Briefly, 1 g of freeze-dried kale powder was weighed into a beaker, followed by the addition of 25 mL of 50 mM acetate buffer (pH 4.0), 0.15 g of α -amylase, and 0.2 g of pepsin, and the mixture was stirred for 10 min. Subsequently, 1 mL of 1% KCN was added, and the solution was stirred for 5 min. The enzyme reaction was activated by incubating the mixture in a water bath (PDWB-22; Lab Partner, Seoul, Korea) at 37 °C for 30 min. It was subsequently terminated by incubating the mixture at 100 °C for 30 min. The solution was cooled to room temperature and centrifuged at

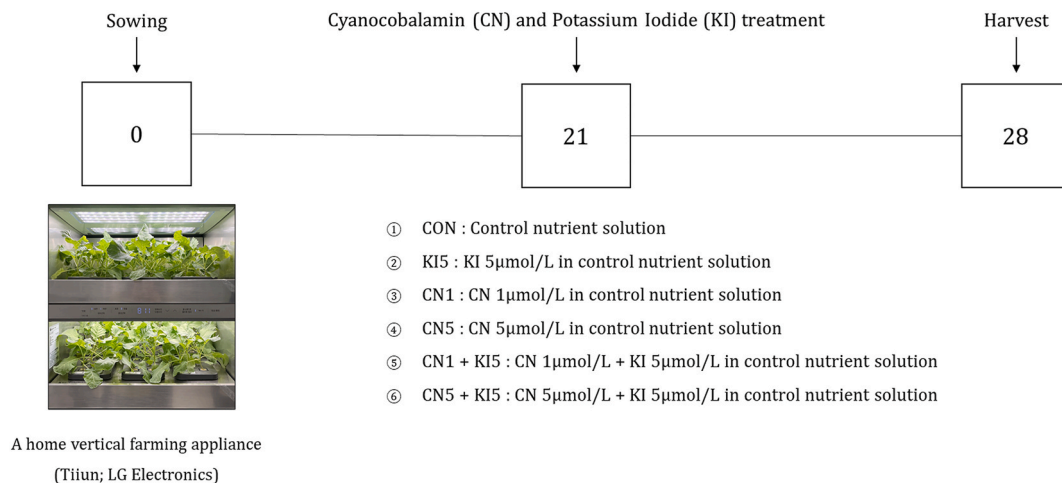


Fig. 1. Illustrations of a home-use vertical farming device and a flowchart of the cultivation procedure.

4 °C and 4000 rpm for 25 min (LaboGene-1248; Gyrozen Co., Seoul, Korea) to collect the supernatant. The pH was adjusted to 7 using acetic acid, and the solution was filtered using filter paper (589/2 Whatman; Whatman, Dassel, Germany). The final extract volume was adjusted to 50 mL using distilled water.

An immunoaffinity column (EASI-EXTRACT® Vitamin B₁₂, product code: R80B; R-Biopharm Rhone Ltd Co., Darmstadt, Germany) was equilibrated at 21–25 °C for 30 min, and the buffer inside the column was removed at a flow rate of 2 mL/min. The column was subsequently activated by passing 3 mL of distilled water through it, followed by loading 12 mL of the sample extract to bind vitamin B₁₂ to the column, while the remaining extract was eluted. The column was washed with 3 mL of distilled water to remove impurities, and moisture was subsequently removed from the column. Thereafter, 3 mL of 100% HPLC-grade methanol was passed through the column to elute the CN. The extracts were completely evaporated at 60 °C using a nitrogen concentrator (TurboVap® LV Sample Concentrator; Biotage Co., Uppsala, Sweden), and the residue was reconstituted in 0.5 mL of distilled water and filtered through a 0.45-μm syringe filter before being transferred to an amber vial.

Chromatographic separation was performed using a reversed-phase HPLC system (Agilent Technologies) equipped with an ACE3 C18 column (4.6 × 150 mm, 3 μm; Avantor Co., Radnor, PA, USA). The column temperature was maintained at 30 °C, and 100 μL of sample was injected. The gradient elution conditions were maintained using water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 0.25 mL/min, with the following gradient conditions: 0 min, 100:0; 11 min, 85:15; 19 min, 75:25; 20 min, 90:10; 26 min, 100:0; and 35 min, 100:0 (A:B). The absorbance spectrum was monitored at 316 nm, and the retention time of CN was 23.22 min.

2.6. Mineral content

The iodine content of the kale samples was analyzed using the method of the Korean Ministry of Food and Drug Safety's Food Code (No. 2021-54; [Safety, 2021]) via inductively coupled plasma mass spectrometry (7800 ICP-MS; Agilent, CA, USA). The zinc, sodium, and potassium contents of the kale samples were determined based on the method described by Son et al. (2021). Mineral content was analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, OPTIMA 5300 DV; PerkinElmer, Waltham, MA, USA) at Chung-Ang University's BT Research Facility Center.

2.7. Color values

Color values were measured by placing 3 g of freeze-dried kale powder in a 35Φ Petri dish and using a color difference meter (Konica Minolta, CM-36dG, Tokyo, Japan). The light source and measurement angle were set to D65-10° (D65-illuminant, 10° observer), and color values (lightness [L*], redness [a*], and yellowness [b*]) were measured using the CIE L*a*b* color system.

2.8. Chlorophyll content

The chlorophyll content of the kale samples was determined using the method described by Wellburn (1994), with certain modifications. Briefly, 1 g of freeze-dried kale powder was extracted with 5 mL of 80% acetone at 40 °C for 1 h, and absorbance was measured at 663 and 646 nm using a spectrophotometer (SpectraMax M2e; Molecular Devices, San Jose, CA, USA). Chlorophyll content was calculated using the following equations: chlorophyll *a* concentration = $12.25 \times A_{663} - 2.79 \times A_{646}$ and chlorophyll *b* concentration = $21.5 \times A_{646} - 5.1 \times A_{663}$ (where "A" represents the absorbance at each wavelength).

2.9. Antioxidant activity

The antioxidant activity of the kale samples was measured based on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity and ferric reducing antioxidant power (FRAP) assay. For sample preparation, 0.15 g of freeze-dried kale powder was mixed with 3 mL of 80% ethanol and extracted via sonication at 40 °C for 40 min (JAC-5020; Kodo Co., Hwaseong, Korea). The mixture was subsequently centrifuged at 3000 rpm for 10 min, and the supernatant was collected. The residue was re-extracted twice with 80% ethanol, and the supernatants were combined. The collected supernatant was filtered through a 0.2-μm syringe filter and evaporated using a vacuum concentrator (SPD, 2030; Thermo Fisher Scientific, Waltham, MA, USA). ABTS radical scavenging activity was determined using the method described by Pyo et al. (2024), while FRAP activity was ascertained according to Benzie and Strain (1996). Each sample's antioxidant activity was compared to a Trolox standard and is expressed as Trolox equivalents (TE). Total flavonoid content (TFC) and total phenolic compounds content (TPC) were determined using the methods of Meda et al. (2005) and Singleton and Rossi (1965), respectively. TFC and TPC are expressed as catechin equivalents (CE) and gallic acid equivalents (GAE).

2.10. Ascorbic acid content

Freeze-dried kale powder (0.15 g) was mixed with 3 mL of 0.1 M acetic acid and extracted under sonication at 15 °C for 10 min. The extract was centrifuged at 4 °C and $10,000 \times g$ for 15 min and the supernatant was filtered through a 0.2- μ m syringe filter. Analysis was performed using a reversed-phase HPLC system (Agilent Technologies, USA) equipped with a C-610 H column (30 cm $\times$ 7.8 mm, 9 μ m; Supelco Co., Bellefonte, PA, USA). The column temperature was maintained at 40 °C, and 20 μ L of sample was injected. The gradient conditions involved the use of 0.1 M perchloric acid as the mobile phase at a flow rate of 0.4 mL/min, with a total run time of 30 min. Ascorbic acid was monitored at 210 nm, with a retention time of 16.41 min.

2.11. Individual phenolic compound content

Individual phenolic compound content was determined according to the methods described by Wang et al. (2013) and Ju et al. (2024), with some modifications. Analysis was performed using a reversed-phase HPLC system (Agilent Infinity Series 1260; Agilent Technologies, USA) equipped with a C18 ODS-AQ column (4.6 $\times$ 150 mm, 5 μ m; YMC, Meridian, ID, USA). The column temperature was maintained at 40 °C, and 20 μ L of sample was injected. Gradient elution was performed using water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B) at a flow rate of 1.0 mL/min, with the following gradient conditions: 0 min, 95:5; 10 min, 90:10; 35 min, 87:13; 45 min, 85:15; 50 min, 82:18; 60 min, 81:19; 70 min, 78:22; 75–76 min, 20:80; and 80–85 min, 95:5 (A:B). The absorbance spectrum was monitored at 254 nm, and each peak was identified using phenolic compound standards.

2.12. Glucosinolate content

The glucosinolate content of the kale samples was ascertained using the method described by Kim et al. (2018), with slight modifications. Briefly, 100 mg of freeze-dried kale powder was mixed with 2 mL of 70% methanol and boiled in a water bath at 90 °C for 10 min. The solvent was cooled on ice for 15 min and centrifuged at 3000 rpm for 15 min to collect the supernatant. The residue was re-extracted with 70% methanol, and the supernatants were combined. Thereafter, 0.1 mL of 1 mM benzyl-glucosinolate was added to the extract and the volume adjusted to 4 mL with 70% methanol. To precipitate proteins, 1.5 mL of the solution was transferred to a microcentrifuge tube (MCT-200-C; Axygen®, Union City, CA, USA), mixed with 0.15 mL of a 1:1 mixture of 1 M lead acetate and 1 M barium acetate, and subsequently centrifuged at $13,000 \times g$ for 5 min. The supernatant (1 mL) was loaded onto an open column filled with DEAE Sephadex A-25 anion exchange resin pre-activated with 0.1 M sodium acetate. The column was sealed, and 0.2 mL of purified arylsulfatase (Sigma-Aldrich, USA) was added, followed by incubation in the dark at room temperature for 18 h. Desulfo-glucosinolates were eluted with distilled water (0.5 mL, twice) and filtered through a 0.2- μ m syringe filter. The prepared samples were analyzed using a reversed-phase HPLC system (Agilent Technologies, USA) equipped with a C18 ODS-AQ column (YMC, USA). The column oven temperature was maintained at 35 °C, and 20 μ L of sample was injected. The elution conditions were maintained using water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 1.0 mL/min, with the following gradient conditions: 0 min, 99.5:0.5; 7 min, 98.5:1.5; 15 min, 90:10; 25 min, 80:20; 35–39 min, 70:30; and 41–45 min, 99.5:0.5 (A:B). The absorbance spectrum was analyzed at 227 nm, and each peak was identified using glucosinolate standards. The glucosinolate content of the kale samples was quantified by comparing the peak area to that of the internal standard, namely, benzyl-glucosinolate.

2.13. Mechanical texture

The mechanical texture of the kale samples was evaluated using a TA-XT ExpressC Texture Analyzer (Stable Micro Systems, Godalming, United Kingdom). Textural characteristics were assessed using two texture analysis methods: puncture and cutting tests. The puncture test was conducted on the leaf section using a P/20 cylindrical probe. The analysis was performed in compression mode, with pre-test, test, and post-test speeds of 5, 1, and 10 mm/s, respectively. The cutting test was conducted on the petiole using an HDP/BS Knife-Edge probe. Compression mode was applied, and the pre-test, test, and post-test speeds were set to 5, 3, and 10 mm/s, respectively. The trigger force was set at 10 g for both tests.

2.14. Sensory analysis

The sensory characteristics of the kale samples were evaluated using quantitative descriptive analysis (QDA). A trained panel of 12 participants (9 women and 3 men) evaluated each attribute of kale using a 15-cm line scale, focusing on seven sensory characteristics: one for appearance (brightness), one for odor (grassy), two for taste (salty, bitter), one for flavor (grassy), one for mouthfeel (astringent), and one for texture (firmness). All panelists were recruited from Chung-Ang University. The entire sensory study design was approved by the Chung-Ang University Institutional Review Board (IRB; Approval No: 1041078-20240304-HR-043; Approval date: May 7, 2024) and conducted in compliance with IRB ethical guidelines. Prior to the sensory analysis, all participants received information regarding the study and the samples, and provided their voluntary consent to participate.

2.15. Statistical analysis

Statistical analysis of the experimental data was performed using IBM SPSS Statistics (version 28; IBM, Armonk, NY, USA). The statistical significance of differences among sample groups was assessed using one-way analysis of variance (ANOVA). Before conducting ANOVA, normality and homogeneity of variances were tested. Duncan's multiple range test was conducted as a post-hoc analysis at a significance level of $p < 0.05$. Experimental results are presented as the mean $\pm$ standard deviation.

3. Results

3.1. Growth and yield of CN- and KI-treated kale samples

To evaluate the effects of CN and KI treatment on kale growth, six growth parameters (leaf number, shoot height, leaf length, leaf width, fresh weight, and dry weight) were measured (Fig. 2). On day 28, no statistically significant differences in leaf number, shoot height, leaf length, or leaf width were noted among all sample groups. Although the fresh weight of the CN1 group was slightly lower than that of the control group (CON), other treatment groups did not exhibit significant yield differences compared with CON.

3.2. CN and mineral contents of CN- and KI-treated kale samples

Combination treatment with CN and KI demonstrated significant differences in uptake efficiency depending on the treatment concentration (Table 1). The application of CN and KI at equal concentrations (CN5+KI5) elicited higher levels of CN ($3.940 \pm 0.323 \mu\text{g/g DW}$) and I ($22.087 \pm 0.804 \mu\text{g/g DW}$) than individual treatment (CN5 and KI5; $p < 0.05$), indicating a synergistic effect between the two components. Conversely, when CN was applied at a lower concentration than KI (CN1+KI5), the accumulation of both components markedly decreased compared with that in the CN1 and KI5 samples ($p < 0.05$). This suggests that the balance between CN and KI potentially plays a crucial role in the

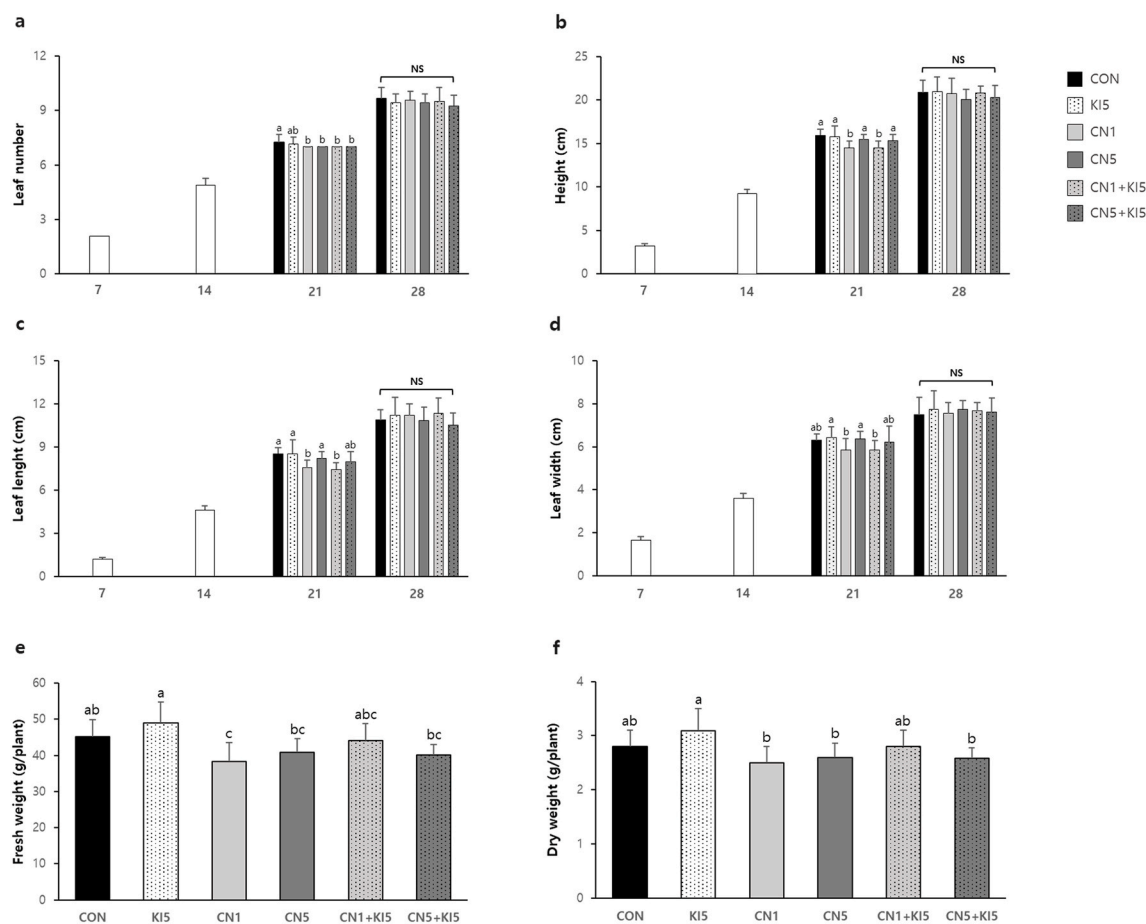


Fig. 2. The growth indicators of cyanocobalamin (CN)- and KI-treated kale samples. Leaf number (a), plant height (b), leaf length (c), leaf width (d), fresh weight (e), and dry weight (f) were determined. Parameters (a)–(d) were measured during the cultivation period once a week using twelve biologically independent plant samples ($n = 12$), while (e) and (f) were assessed postharvest on day 28 after sowing using six biologically independent plant samples ($n = 6$). Data are presented as the mean $\pm$ standard deviation. Different letters within a group indicate statistically significant differences ($p < 0.05$). “NS” denotes no statistically significant differences. Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at 1 $\mu\text{mol/L}$ (CN1) or 5 $\mu\text{mol/L}$ (CN5), KI at 5 $\mu\text{mol/L}$ (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

Table 1

Cyanocobalamin (CN) and mineral contents of kale samples treated with CN and KI.

Sample	Cyanocobalamin ($\mu\text{g}/100 \text{ g dry weight}$)	I ($\mu\text{g}/\text{g dry weight}$)	Zn ($\mu\text{g}/\text{g dry weight}$)	Na ($\text{mg}/\text{g dry weight}$)	K ($\text{mg}/\text{g dry weight}$)
CON	ND	ND	66.33 ± 5.74^{bc}	7.87 ± 0.73^{bc}	173.97 ± 5.68^{ab}
KI5	ND	16.47 ± 0.15^b	88.00 ± 3.74^{ab}	9.39 ± 0.54^b	154.40 ± 4.81^c
CN1	56.13 ± 10.26^c	ND	61.00 ± 2.45^c	8.41 ± 0.24^{bc}	162.73 ± 6.34^{bc}
CN5	266.79 ± 26.48^b	ND	101.67 ± 8.18^a	10.91 ± 0.44^a	178.47 ± 1.27^a
CN1+KI5	3.68 ± 0.51^d	0.25 ± 0.01^c	82.33 ± 17.46^{abc}	7.87 ± 1.31^{bc}	163.90 ± 5.77^{bc}
CN5+KI5	394.04 ± 32.30^a	22.09 ± 0.80^a	102.67 ± 17.25^a	7.56 ± 0.33^c	170.50 ± 4.54^{ab}

Data are presented as the mean $\pm$ standard deviation ($n = 3$, representing three biologically independent pooled samples).

Different letters within the same column indicate a significant difference at $p < 0.05$.

“ND” indicates that the values were not detected.

Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at 1 $\mu\text{mol/L}$ (CN1) or 5 $\mu\text{mol/L}$ (CN5), KI at 5 $\mu\text{mol/L}$ (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

nutrient uptake of kale.

Significant differences in Zn, Na, and K content were also observed among the groups. The Zn content significantly increased in the CN5 ($101.67 \pm 8.18 \mu\text{g/g DW}$) and CN5+KI5 ($102.67 \pm 17.25 \mu\text{g/g DW}$) groups compared with that in CON ($66.33 \pm 5.73 \mu\text{g/g DW}$; $p < 0.05$), suggesting that CN treatment potentially elevates Zn content in kale. The Na content was significantly higher in the CN5 treatment group ($10.91 \pm 0.44 \text{ mg/g DW}$) than in CON ($7.87 \pm 0.73 \text{ mg/g DW}$; $p < 0.05$), while the other four treatment groups (KI5, CN1, CN1+KI5, and CN5+KI5) did not exhibit significant differences in Na content from

CON. A significant reduction in K content was observed in the KI5 group compared with that in CON ($p < 0.05$). Other samples did not display significant differences in K content compared with CON.

3.3. Color values and chlorophyll content of CN- and KI-treated kale samples

The color values and chlorophyll content of the CN- and KI-treated kale samples are presented in Table 2. CON (53.68 ± 0.18) did not exhibit significant L^* value differences compared with the treatment

Table 2

Color values and chlorophyll content of kale samples treated with cyanocobalamin (CN) and KI.

Sample	Color values			Chlorophyll (mg/g dry weight)		
	L (lightness)	a (redness)	b (yellowness)	Chlorophyll a	Chlorophyll b	Chlorophyll (a+b)
CON	53.68 ± 0.18 ^{NS}	−7.06 ± 0.10 ^b	15.08 ± 0.32 ^a	1.343 ± 0.016 ^a	1.318 ± 0.030 ^a	2.660 ± 0.034 ^a
KI5	51.82 ± 0.23	−6.52 ± 0.24 ^a	13.91 ± 0.50 ^b	1.329 ± 0.004 ^{ab}	1.237 ± 0.044 ^c	2.557 ± 0.043 ^c
CN1	52.15 ± 0.11	−6.84 ± 0.24 ^b	14.78 ± 0.45 ^a	1.339 ± 0.025 ^{ab}	1.278 ± 0.034 ^b	2.617 ± 0.028 ^b
CN5	52.24 ± 0.13	−6.46 ± 0.10 ^a	13.81 ± 0.17 ^b	1.335 ± 0.003 ^{ab}	1.263 ± 0.027 ^{bc}	2.597 ± 0.028 ^{bc}
CN1+KI5	51.72 ± 0.13	−6.55 ± 0.30 ^a	14.02 ± 0.67 ^b	1.325 ± 0.004 ^b	1.288 ± 0.025 ^{ab}	2.613 ± 0.028 ^b
CN5+KI5	51.66 ± 0.25	−6.45 ± 0.06 ^a	13.86 ± 0.41 ^b	1.335 ± 0.002 ^{ab}	1.254 ± 0.024 ^{bc}	2.589 ± 0.024 ^{bc}

Data are presented as the mean ± standard deviation (n = 6 for color values and n = 9 for chlorophyll content, representing six or nine biologically independent plant samples).

The presence of different letters within the same column indicates a statistically significant difference at $p < 0.05$.

“NS” denotes no statistically significant differences.

Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at 1 µmol/L (CN1) or 5 µmol/L (CN5), KI at 5 µmol/L (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

groups. Besides CN1, all treatment groups yielded significant a^* value increases compared with CON ($p < 0.05$), and the highest a^* value was observed in the CN5+KI5 treatment group (-6.45 ± 0.06). All treatment groups, except the CN1 group, yielded significantly decreased b^* values compared with CON ($p < 0.05$).

In terms of chlorophyll content, the chlorophyll a content of CON was 1.343 ± 0.016 mg/g DW. The CN1+KI5 treatment group yielded a chlorophyll a content of 1.325 ± 0.004 mg/g DW, displaying a significant reduction compared with CON ($p < 0.05$). However, the other four treatment groups (KI5, CN1, CN5, and CN5+KI5) exhibited no significant differences compared with CON, suggesting that these treatments did not distinctly affect chlorophyll a content. CON yielded the highest chlorophyll b content (1.318 ± 0.030 mg/g DW), while the KI5 treatment group exhibited the lowest (1.237 ± 0.044 mg/g DW), significantly differing from CON ($p < 0.05$). CON also generated the highest total chlorophyll content ($a + b$) (2.660 ± 0.034 mg/g DW), while all treatment groups yielded significantly low amounts ($p < 0.05$). However, the total chlorophyll content reduction in the treatment groups compared with that in CON was approximately 2–4%.

3.4. Antioxidative capacities of CN- and KI-treated kale samples

The antioxidant capacity of the CN- and KI-treated kale samples was evaluated based on TPC, TFC, ascorbic acid content, ABTS radical scavenging activity, and FRAP activity (Fig. 3). No significant differences in TPC occurred between CON and the CN5+KI5 treatment group (Fig. 3a). Nevertheless, the TPC of the CN1+KI5 treatment group was significantly lower than that of CON ($p < 0.05$). The TFC of CON was 10.88 ± 0.23 mg CE/g DW, a figure similar to that of the CN1 and CN5+KI5 treatment groups (Fig. 3b). However, significant reductions in TFC were observed in three treatment groups (KI5, CN5, and CN1+KI5). The ascorbic acid content of the CN1 and CN5+KI5 treatment groups did not significantly differ from that of CON, while significantly lower levels were detected in the other three groups (KI5, CN5, and CN1+KI5) (Fig. 3c). CON exhibited the highest ABTS radical scavenging activity (9.60 ± 0.17 mg TE/g DW), with a significant decrease observed across all treatment groups (Fig. 3d; $p < 0.05$). The FRAP activity of the CN1 treatment group did not exhibit a significant difference from that of CON (9.80 ± 0.47 and 10.07 ± 0.41 mg TE/g DW, respectively), whereas the other four treatment groups displayed significantly lower activity ($p < 0.05$), ranging from 8.44 ± 0.43 to 8.98 ± 0.34 mg TE/g DW (Fig. 3e).

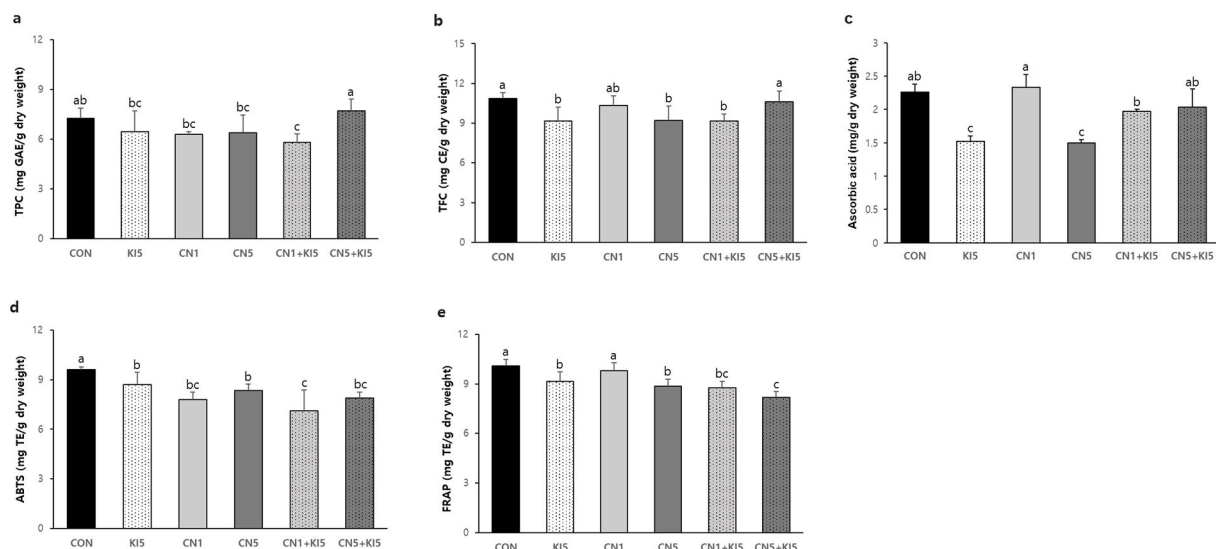


Fig. 3. Antioxidant capacities of cyanocobalamin (CN)- and KI-treated kale samples. Total phenolic content (TPC) (a), total flavonoid content (TFC) (b), ascorbic acid content (c), ABTS radical scavenging activity (d), and FRAP activity (e) were measured. Data are presented as the mean ± standard deviation (n = 3, representing three biologically independent pooled samples). The extraction was performed in triplicate for each sample, and each extract was analyzed in duplicate. Different letters indicate statistically significant differences within each parameter ($p < 0.05$). “NS” denotes no statistically significant differences. Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at 1 µmol/L (CN1) or 5 µmol/L (CN5), KI at 5 µmol/L (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

3.5. Individual phenolic compound contents of CN- and KI-treated kale samples

The phenolic compound analysis results of the CN- and KI-treated kale samples are presented in Fig. 4. No significant differences in the contents of neochlorogenic acid, 3,4-dicaffeoylquinic acid (3,4-DCQA), and 3,5-dicaffeoylquinic acid (3,5-DCQA) were identified among all sample groups. The CN5+KI5 treatment group exhibited the highest amount of chlorogenic acid ($253.22 \pm 15.96 \mu\text{g/g DW}$), whereas the KI5 treatment group yielded the lowest (Fig. 4b). The p-coumaric acid content significantly increased in three treatment groups (KI5, CN5, and CN5+KI5) compared with that in CON, with values ranging from 26.89 ± 0.90 to $28.20 \pm 0.87 \mu\text{g/g DW}$ ($p < 0.05$). The CN5+KI5 group also yielded a significantly elevated 3,5-DCQA content ($113.19 \pm 4.28 \mu\text{g/g DW}$) compared with CON ($63.3 \pm 6.28 \mu\text{g/g DW}$).

3.6. Glucosinolate content of CN- and KI-treated kale samples

To quantify the glucosinolate content of the CN- and KI-treated kale samples, eight glucosinolate standards (glucoiberin, progoitrin, sinigrin, glucoraphanin, gluconapin, glucobrassicin, gluconasturtiin, and neoglucobrassicin) were employed; however, only four glucosinolate types (sinigrin, gluconapin, glucobrassicin, and gluconasturtiin) were detected in the kale samples (Fig. 5). The gluconapin content exhibited a significant increase in the KI5 and CN5 groups (3.26 ± 0.30 and $3.58 \pm 0.12 \mu\text{mol/g DW}$, respectively) compared with that in CON ($p < 0.05$). CN1 exhibited the highest glucobrassicin content ($1.83 \pm 0.27 \mu\text{mol/g DW}$); nonetheless, no significant differences were noted compared with CON. CON yielded the highest gluconasturtiin content ($1.49 \pm 0.26 \mu\text{mol/g DW}$), while the CN5 treatment group exhibited a slight decrease thereof ($1.05 \pm 0.07 \mu\text{mol/g DW}$). The sinigrin and total glucosinolate contents did not exhibit significant differences across all samples.

3.7. Mechanical texture and sensory analysis of CN- and KI-treated kale samples

The mechanical texture of the kale samples was evaluated using

puncture (leaf) and cutting (petiole) tests, and no significant differences were observed across all groups, with values ranging from 33.67 to 36.67 g and 215.33 to 218.17 g, respectively. To assess the sensory characteristics of the CN- and KI-treated kales, seven sensory attributes were evaluated using the QDA method (Table 3). No significant differences in five attributes (grassy odor, saltiness, bitterness, grassy flavor, and astringency) were observed among the sample groups. CON yielded the highest brightness value (8.25 ± 0.94), while the CN5+KI5 treatment group recorded the lowest (6.02 ± 2.05 ; $p < 0.05$). The CN1+KI5 treatment group exhibited the highest firmness value (6.37 ± 2.07), whereas the KI5 treatment group yielded the lowest (4.64 ± 1.27). However, both samples did not display significant differences compared with CON.

4. Discussion

Vitamin B₁₂ is an essential water-soluble micronutrient involved in enzymatic reactions, including DNA synthesis and inorganic carbon assimilation, ultimately influencing gene regulation, hematopoiesis, and nervous system metabolism (Kumar et al., 2023; Oh et al., 2021). Populations at high risk of vitamin B₁₂ deficiency include older adults, children, women of reproductive age, patients with autoimmune conditions (e.g., pernicious anemia and atrophic gastritis), and individuals with impaired cobalamin absorption, such as those following strict vegetarian or vegan diets (Stabler, 2020). Vitamin B₁₂ deficiency not only causes megaloblastic anemia, characterized by the presence of abnormal, nucleated red blood cells, and neurological disorders but is also frequently accompanied by deficiencies of other micronutrients, including zinc, folate, iron, and protein (Health, 2021; Stabler, 2020).

Generally, plants neither synthesize nor require vitamin B₁₂; thus, they lack metabolic pathways where vitamin B₁₂ serves as a coenzyme (Kumar et al., 2023; Watanabe and Bito, 2018). While previous research has revealed interactions between vitamin B₁₂ and metal-ion absorption in animal and microbial metabolism (Banerjee and Ragsdale, 2003; Osman et al., 2021), the specific mechanisms underlying vitamin B₁₂ uptake in plants remain largely unexplored (Oh et al., 2021). Possible uptake pathways may include non-specific transport, passive diffusion, or interactions with root-associated microorganisms; however, these

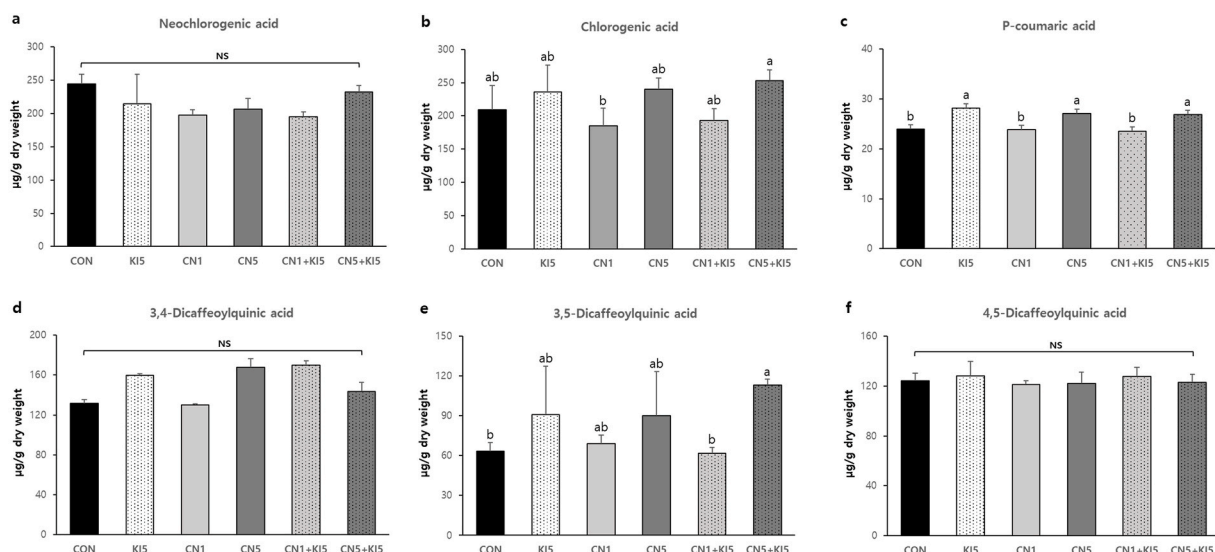


Fig. 4. Individual phenolic compound contents of cyanocobalamin (CN)- and KI-treated kale samples. The contents of neochlorogenic acid (a), chlorogenic acid (b), p-coumaric acid (c), 3,4-dicaffeoylquinic acid (d), 3,5-dicaffeoylquinic acid (e), and 4,5-dicaffeoylquinic acid (f) were quantified via HPLC. Data are presented as the mean $\pm$ standard deviation ($n = 3$, representing three biologically independent pooled samples). The extraction was performed in triplicate for each sample, and each extract was analyzed. Different letters indicate significant differences within a group ($p < 0.05$). "NS" denotes no statistically significant differences. Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at $1 \mu\text{mol/L}$ (CN1) or $5 \mu\text{mol/L}$ (CN5), KI at $5 \mu\text{mol/L}$ (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

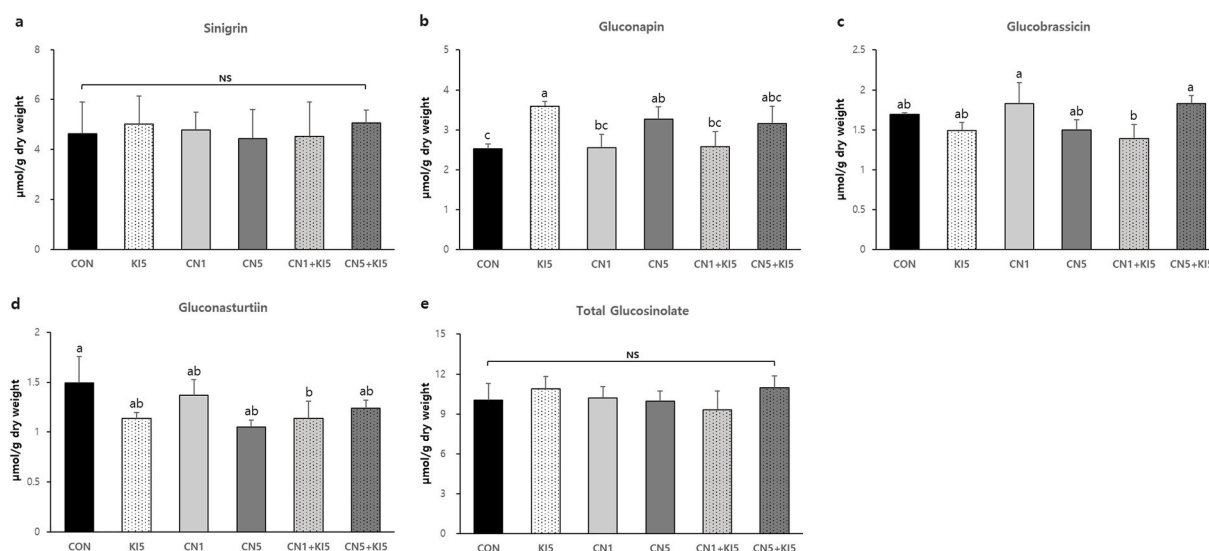


Fig. 5. Glucosinolate content of cyanocobalamin (CN)- and KI-treated kale samples. The contents of sinigrin (a), gluconapin (b), glucobrassicin (c), gluconasturtiin (d), and total glucosinolate (e) were quantified using HPLC. Data are presented as the mean $\pm$ standard deviation ($n = 3$, representing biologically independent pooled samples). The extraction was performed in triplicate for each sample, and each extract was analyzed. Different letters indicate significant differences within each group ($p < 0.05$). “NS” denotes no statistically significant differences. Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at 1 $\mu\text{mol/L}$ (CN1) or 5 $\mu\text{mol/L}$ (CN5), KI at 5 $\mu\text{mol/L}$ (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

Table 3

Mechanical texture properties and sensory characteristics of kale samples treated with cyanocobalamin (CN) and KI.

	Texture (g)		Sensory characteristics						
	Punching	Cutting	Brightness	Grassy odor	Saltiness	Bitterness	Grassy flavor	Astringency	Firmness
CON	36.67 $\pm$ 3.14 ^{NS}	218.17 $\pm$ 27.27 ^{NS}	8.25 $\pm$ 0.94 ^a	1.71 $\pm$ 0.73 ^{NS}	4.31 $\pm$ 2.21 ^{NS}	5.83 $\pm$ 2.32 ^{NS}	6.95 $\pm$ 2.72 ^{NS}	6.56 $\pm$ 2.01 ^{NS}	5.59 $\pm$ 1.91 ^{ab}
KI5	36.50 $\pm$ 2.99	216.00 $\pm$ 25.16	7.13 $\pm$ 1.66 ^{abc}	1.38 $\pm$ 0.70	3.48 $\pm$ 2.36	5.31 $\pm$ 1.69	7.01 $\pm$ 1.98	6.37 $\pm$ 1.43	4.64 $\pm$ 1.27 ^b
CN1	34.50 $\pm$ 4.82	219.00 $\pm$ 30.99	7.64 $\pm$ 1.46 ^{ab}	1.98 $\pm$ 1.11	3.97 $\pm$ 1.44	4.58 $\pm$ 1.89	6.88 $\pm$ 2.46	5.98 $\pm$ 1.81	6.09 $\pm$ 2.05 ^{ab}
CN5	33.67 $\pm$ 2.92	215.33 $\pm$ 27.53	6.22 $\pm$ 1.87 ^{bc}	1.41 $\pm$ 0.66	3.65 $\pm$ 2.19	5.66 $\pm$ 1.54	7.46 $\pm$ 2.22	7.35 $\pm$ 1.52	5.23 $\pm$ 1.46 ^{ab}
CN1+KI5	36.50 $\pm$ 6.10	221.33 $\pm$ 59.99	7.44 $\pm$ 1.32 ^{abc}	1.68 $\pm$ 0.55	4.60 $\pm$ 1.80	5.73 $\pm$ 2.08	7.08 $\pm$ 2.15	6.53 $\pm$ 2.08	6.37 $\pm$ 2.07 ^a
CN5+KI5	35.50 $\pm$ 1.89	217.50 $\pm$ 47.84	6.02 $\pm$ 2.05 ^c	1.28 $\pm$ 0.63	3.28 $\pm$ 2.16	4.86 $\pm$ 2.01	7.01 $\pm$ 2.12	6.93 $\pm$ 1.49	4.81 $\pm$ 1.35 ^{ab}

Data are presented as the mean $\pm$ standard deviation.

Mechanical texture analysis was performed using six biologically independent plant samples ($n = 6$).

Sensory characteristics were evaluated by a trained panel of 12 participants using quantitative descriptive analysis.

The presence of different letters within the same column indicates a statistically significant difference at $p < 0.05$.

“NS” denotes no statistically significant differences.

Kale plants were treated with a control nutrient solution (CON) or nutrient solutions containing CN at 1 $\mu\text{mol/L}$ (CN1) or 5 $\mu\text{mol/L}$ (CN5), KI at 5 $\mu\text{mol/L}$ (KI5), or combinations of CN and KI (CN1+KI5 and CN5+KI5).

possibilities remain speculative due to the limited understanding of vitamin B₁₂ transport in plant systems. In addition, interactions with coexisting inorganic ions such as iodine may influence the transport, stability, or binding forms of vitamin B₁₂. Although this study has limitations in elucidating the underlying mechanisms, our results demonstrate that vitamin B₁₂ and iodine can effectively accumulate in kale. Moreover, because CN- or KI-containing nutrient solutions were supplied to the roots and these nutrients were distributed to the above-ground parts of kale, the absorption and translocation of these nutrients in kale were demonstrated. The individual CN-treatment groups (CN1 and CN5) exhibited a positive correlation between treatment concentration and accumulation rate in kale, displaying consistency with previous studies (Bito et al., 2013; Sato et al., 2004). The recommended daily allowance (RDA) of vitamin B₁₂ for adults is approximately 2.4 μg (Pawlak et al., 2013a, 2013b), and its criteria were fulfilled when 42.86 and 8.99 g of fresh CN1 and CN5 was consumed, respectively. These results suggest that CN-fortified kale may serve as a potential dietary source of vitamin B₁₂, particularly for vegan and vegetarian populations at risk of deficiency.

Dietary iodine supplementation has been attempted globally owing to the prevalence of iodine deficiency, and iodine addition to salt

remains a popular approach (Gonzali et al., 2017). However, reduced salt intake is increasingly recommended to prevent hypertension and cardiovascular diseases (Izydorczyk et al., 2021), suggesting that reliance on iodized salt alone may be insufficient to meet iodine requirements (Zhang et al., 2023). In this context, crop biofortification has emerged as an effective strategy to enhance iodine intake through diet. Notably, iodine, along with vitamin B₁₂, is one of the most deficient micronutrients in the vegan population (Eveleigh et al., 2023). In this study, kale absorbed sufficient amounts of iodine from the nutrient solution. Resultantly, consuming 91.07 g of fresh kale treated with KI5 or 67.90 g of kale treated with CN5+KI5 potentially meets the adult RDA of 150 μg of iodine (Eveleigh et al., 2023). In addition, recent studies have indicated that iodine biofortification could influence plant metabolism and defense mechanisms, potentially through the modulation of reactive oxygen species and antioxidant pathways (Halka et al., 2020; Zhang et al., 2023). We also found that treatment with CN and KI altered the levels of some secondary metabolites in kale; however, the underlying mechanisms still require further investigation.

In the present study, combination treatment with CN and KI appeared to induce more complex interactions in kale compared with individual treatment. When the CN concentration was lower than that of

KI (CN1+KI5), an antagonistic effect on absorption occurred, significantly reducing the accumulation rates of both components in kale. This antagonism suggests competitive interactions between the uptake pathways of specific micronutrients. In contrast, when CN and KI were coadministered at equal concentrations (CN5+KI5), both components exhibited mounted accumulation rates compared with their independent applications, indicating a synergistic effect. These findings suggest interactions between micronutrient uptake pathways that may alter accumulation patterns. Although further investigations at the cellular and molecular levels are required to elucidate the underlying mechanisms of CN and KI uptake and metabolism in plants, these results suggest that multi-biofortification strategies may provide greater metabolic flexibility than traditional single-biofortification approaches. De Zanetti and Van Der Straeten (2024) emphasized the need for an integrated understanding of metabolic interactions between vitamins and other nutrients, beyond simply addressing nutrient deficiencies. Our findings demonstrate that combination treatment with CN and KI potentially induces metabolic alterations beyond simple accumulation, providing deeper insight into its effects on plant metabolism and physiological responses. This connotes the potential of multi-biofortification to activate certain metabolic pathways and regulate the absorption and synthesis of chemical compounds in plants.

This study also demonstrated a significant increase in Zn content through kale biofortification with CN and KI. In particular, Zn content significantly increased in both the CN5 and CN5+KI5 treatment groups, indicating that certain concentrations of CN may positively affect Zn uptake and accumulation in kale. Kieninger et al. (2019) proposed that Zn analogs potentially serve as functional substitutes for vitamin B₁₂, suggesting two possibilities: (1) potential chemical and structural interactions between vitamin B₁₂ and Zn and (2) the enhancement of Zn uptake pathways or metabolic stabilization of Zn by vitamin B₁₂. These findings further indicate that when CN and KI are coadministered, they may act as enzyme cofactors or metabolic promoters, offering a new perspective on micronutrient interactions. Although limited research exists on the direct correlation among vitamin B₁₂, iodine, and Zn content, our study shows that CN and KI biofortification can increase Zn content.

Meanwhile, treatment with CN and KI can affect antioxidant activity and secondary metabolite synthesis in kale. Although TPC and TFC significantly decreased in the CN1+KI5 group, the CN5+KI5 treatment group exhibited similar TPC and TFC levels with CON. The ascorbic acid content significantly decreased in the KI5 treatment group, exhibiting consistency with the findings of Halka et al. (2020), who reported that KI biofortification in tomatoes reduced ascorbic and dehydroascorbic acid levels and attenuated ascorbate peroxidase activity. Our study suggests that combination treatment with CN and KI at a suitable concentration may mitigate these decreases.

Regarding phenolic compounds, the CN5+KI5 treatment group exhibited significantly increased levels of p-coumaric acid and 3,5-DCQA compared with CON. This indicates that combination treatment with CN and KI may promote the synthesis or mitigate the degradation of specific phenolic compounds. Previous studies have shown that exogenous vitamin B treatment enhances phenolic biosynthesis and alleviates oxidative stress-induced growth inhibition (Burguires et al., 2007). Similarly, Keshavarz and Moghadam (2017) suggested that CN treatment may induce endogenous phenolic compounds in soybeans. Therefore, the observed increase in phenolic compounds may reflect the effect of CN and KI treatments in regulating their synthesis or degradation, consistent with previous studies reporting altered levels of secondary metabolites in plants treated with elicitors (Isah, 2019; Ju et al., 2024; Son et al., 2021).

Although multi-biofortification using appropriate levels of CN and KI in kale modified certain biological conditions, it did not negatively impact the growth, visual quality, or sensory traits of kale. Previous studies have demonstrated that independent treatment with suitable concentrations of vitamin B₁₂ and iodine does not affect crop growth and

yield (Bito et al., 2013; Halka et al., 2020; Sato et al., 2004; Yamamoto et al., 2023). However, a review study by Zhang et al. (2023) on iodine-biofortified crops reported that high concentrations of iodine can hinder plant growth and negatively affect physiological conditions. In our study, we applied iodine at a concentration of 5 $\mu\text{mol/L}$, confirming that this level neither negatively affects plant growth or development nor exerts negative impacts when combined with CN. The result of sensory evaluation indicated that CN and KI treatments resulted in slight differences in brightness and firmness; however, no significant changes were observed in flavor, aroma, texture, or overall acceptability. These results suggest that the treatments did not adversely affect the overall sensory quality of kale. In addition, we did not observe notable changes in color values and mechanical texture of kale following CN and KI administration. Therefore, CN–KI multi-biofortified kale may represent a promising approach for enhancing micronutrient content while maintaining acceptable quality characteristics for consumers.

Recent global challenges, including climate change, geopolitical instability, and disruptions in food supply chains, can lead to food security and nutritional imbalance issues (Galanakis, 2023). Occasionally, nutritional imbalances are also observed in well-developed countries due to poor dietary habits or personal beliefs, such as veganism. In this context, the multi-biofortification approach in plant sources provides a potential strategy for delivering essential nutrients to people who suffer from deficiencies and may contribute to the development of sustainable food systems. Vertical farming is a novel agricultural system that can be applied to the production of nutrient-enriched crops due to its well-controlled environmental conditions and easier elicitor application (Tessema et al., 2026). Moreover, the use of vertical farming systems can overcome geographic or environmental constraints (Bouis et al., 2024). Due to these advantages, the improvement of functional compound content in plants using vertical farming has received increasing attention not only in the food industry but for potential applications in cosmetics and medicinal products. Therefore, we believe that the findings of this study could expand academic understanding of nutrient biofortification in plants using vertical farming systems. However, there are some limitations. Only a single crop species was tested in this study as a model plant, and long-term stability during postharvest storage was not evaluated. In addition, the bioavailability of micronutrients derived from fortified plants remains to be clarified. Thus, future research should focus on evaluating the bioavailability of these micronutrients to determine their actual efficacy after consumption. Furthermore, developing multi-biofortification strategies that consider interactions among vitamin B₁₂, iodine, and other micronutrients may offer a promising approach to addressing micronutrient deficiencies, including hidden hunger.

5. Conclusion

This study aimed to analyze the effects of multi-biofortification with vitamin B₁₂ and iodine on the growth parameters, nutritional quality, chemical constituents' contents, antioxidant activity, and sensory properties of kale. The accumulation efficiency of CN and KI was significantly influenced by their respective concentrations and ratios. The independent CN-treatment groups (CN1 and CN5) exhibited a positive correlation between CN concentration and accumulation rate; however, antagonistic and complementary effects were observed following combination treatment with CN and KI. When the CN concentration was lower than that of KI (CN1+KI5), an antagonistic effect occurred, leading to a significant decrease in the accumulation of both components. In contrast, when CN and KI were coadministered at equal concentrations (CN5+KI5), a synergistic effect was observed, significantly increasing the accumulation of both components. Biofortification with CN and KI not only elevated CN and iodine contents but also increased the Zn content, and the CN5+KI5 treatment group exhibited increased amounts of certain phenolic compounds, such as 3,5-DCQA and p-coumaric acid. This study also confirmed that multi-

biofortification with CN and iodine did not negatively affect the growth, quality, or consumer acceptability of kale. Collectively, multi-biofortification of kale with CN and KI is a potentially promising strategy for enhancing nutritional quality, offering a means of addressing nutrient deficiencies among vulnerable populations. Future studies should focus on optimizing the concentrations and ratios of vitamin B₁₂ and iodine and evaluating environmental conditions to develop more effective multi-biofortification strategies for public health nutrition.

CRedit authorship contribution statement

S.L., M.B., and Y.J.S. contributed to the conceptualization of the research design. S.L., M.B., Y.J.P., Y.S., S.A.K., and C.R.M. conducted experimental analyses under the supervision of Y.J.S. S.L. and Y.J.S. wrote and revised the original draft. All authors reviewed the manuscript and approved for publication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets are available from the corresponding author on reasonable request.

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