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Hydroponic Production of Reduced-Potassium Swiss Chard and Spinach: A Feasible Agronomic Approach to Tailoring Vegetables for Chronic Kidney Disease Patients

Massimiliano D'Imperio, Francesco F. Montesano *, Massimiliano Renna, Angelo Parente, Antonio F. Logrieco and Francesco Serio *

Institute of Sciences of Food Production, CNR–National Research Council of Italy, Via Amendola 122/D, 70126 Bari, Italy; massimiliano.dimperio@ispa.cnr.it (M.D.I.); massimiliano.renna@ispa.cnr.it (M.R.); angelo.parente@ispa.cnr.it (A.P.); antonio.logrieco@ispa.cnr.it (A.F.L.)

* Correspondence: francesco.montesano@ispa.cnr.it (F.F.M.); francesco.serio@ispa.cnr.it (F.S.); Tel.: +39-080-5929-385 (F.F.M. & F.S.)

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Abstract: Tailored foods are specifically suitable for target groups of people with particular nutritional needs. Although most research on tailored foods has been focused on increasing the nutrient content in plant tissues (biofortification), in populations with specific physiological conditions, it is recommended to reduce the uptake of specific nutrients in order to improve their health. People affected by chronic kidney disease (CKD) must limit their consumption of vegetables because of the generally high potassium (K) content in the edible parts. This study aimed to define an appropriate production technique for two baby leaf vegetables, spinach (*Spinacia oleracea* L.) and Swiss chard (*Beta vulgaris* L. ssp. *vulgaris*), with reduced K tissue content, minimizing the negative effects on their crop performance and overall nutritional quality. Plants were grown in a hydroponic floating system. The K concentration in the nutrient solution (NS) was reduced from 200 mg/L (K_{200}, the concentration usually used for growing baby leaf vegetables in hydroponic conditions) to 50 mg/L over the entire growing cycle (K_{50}) or only during the seven days before harvest ($K_{50\text{-}7d}$). The reduction of K in the NS resulted in a significant decrease of K tissue content in both species (32% for K_{50} and 10% for $K_{50\text{-}7d}$, on average), while it did not, in general, compromise the crop performance and quality traits or the bioaccessibility of K, magnesium, and calcium. The production of reduced-potassium leafy vegetables is a feasible tailored nutrition approach for CKD patients in order to take advantage of the positive effects of vegetable consumption on health without excessively increasing potassium intake.

Keywords: *Spinacia oleracea* L.; *Beta vulgaris* L. ssp. *vulgaris*; soilless cultivation; tailored food; potassium bioaccessibility; potassium intake

1. Introduction

A novel challenge in agriculture is the production of tailored foods, i.e., foods specifically suitable for target groups of people with particular nutritional needs. In fact, in recent years, a number of studies have highlighted the possibility of producing vegetables for specific physiological conditions, such as biofortified vegetables, with the aim of counteracting different nutritional deficits [1–8]. In general, these authors reported evidence on the use of specific growing protocols aimed to increase the content of specific nutrients in plant tissues, such as iodine (I), silicon (Si), calcium (Ca), selenium (Se), zinc (Zn), and iron (Fe). However, although most research on tailored foods has been focused on biofortification in order to increase the content of nutrients in plant tissues, it should be noted that in populations with specific physiological conditions, it is recommended to reduce the uptake of specific nutrients in order to improve their health condition. An example of this is reducing potassium (K) and sodium (Na) intake for chronic kidney disease (CKD) patients in order to improve their physiological condition. Chronic kidney disease is defined as a condition of impaired renal function [9]. Epidemiological data show that CKD is a widespread disease with an increasing trend in the world population. It is estimated that about 10% of the worldwide population is affected by CKD, and millions die each year because they do not have access to affordable treatments [10]. Nutritional approaches play an important role in improving the physiological condition of these patients. In order to prevent the occurrence of hyperkalemia (i.e., K level in the blood higher than normal), it is recommended to avoid eating foods with high levels of K, including fruits and vegetables. Vegetables, in fact, are generally rich in K; higher levels are present in leafy vegetables such as spinach (5580 mg/kg of fresh weight) and Swiss chard (3790 mg/kg of fresh weight) [11,12] This element constitutes up to 10% of plant dry weight and is considered a macronutrient essential for plants, with fundamental effects on their health, growth, and development [13]. As a result, it is difficult to reduce the physiological K concentration in plants without having detrimental effects on yield and marketable quality, because of the fundamental physiological functions of K, including enzyme activation, osmotic regulation, photosynthesis, and translocation of the products of photosynthesis [14,15]. Recently, Renna et al. (2018) [16] reported reduction of K tissue concentration in microgreens of two cultivars of chicory (*Cichorium intybus* L.) and one cultivar of lettuce (*Lactuca sativa* L. group crispa). However, microgreens are unconventional vegetables, considered niche products generally accessible only to restricted groups of people. Unlike microgreen cultivation, characterized by a short growing cycle (generally 20 days from germination), reduction of K in conventional vegetables such as baby leaf vegetables, with plants cultivated over a complete growing cycle to be ready for selling, is more difficult. Both (i) the extent of the reduced level of K available to plants as supplied in the fertilization program and (ii) the time of exposure to K deprivation have been reported to negatively affect market quality and yield [13] and/or nutritional quality (i.e., ion content), generally with an increase of Na content in vegetables [17].

Among different cultivation techniques, a floating hydroponic system, which involves growing plants on trays floating in tanks filled with a nutrient solution (NS), has proven to be an interesting tool to obtain baby leaf vegetables with modified tissue concentrations of specific minerals (Ca and Si) [2–4] in edible parts of the plants. By acting on the mineral composition of the NS, it is possible to modify to a certain extent the tissue concentration of target ions. The objectives of this study were as follows: (i) To define a cultivation protocol suitable to produce baby leaf vegetables (spinach and Swiss chard) with low K tissue content, without negatively affecting plant growth and marketable quality, with a main focus on the application of the technique; (ii) to verify possible interactions of reduced K tissue concentration with the content of Ca and Mg, important factors for the nutritional needs of CKD patients, and oxalate, an important antinutritional compound, in the edible parts of plants; and (iii) to assess the ion bioaccessibility of these products by using an in vitro gastrointestinal digestion process.

We hypothesized that a floating hydroponic system with reduced concentration of K in the NS compared to the typical level for hydroponic production of baby leaf vegetables (200 mg/L) [3] could be adopted to obtain tailored baby leaf vegetables for CKD patients with low K tissue content, satisfactory levels and bioaccessibility of other important nutrients (Ca and Mg), and no increase of oxalate.

2. Materials and Methods

2.1. Plant Materials and Experimental Conditions

Experiments were carried out from 11 November 2016 to 28 February 2017 in a plastic greenhouse at the La Noria experimental farm of the Institute of Sciences of Food Production (CNR-ISPA) in Mola di Bari (BA), Southern Italy (41°03′ N, 17°04′ E; 24 m a.s.l.). Plant material details and dates of the experiment, and main climatic conditions during the growing cycle are reported in Table 1 and Figure 1, respectively. Spinach and Swiss chard seeds were sown in cell trays containing peat.

After the seedlings reached the 2 true leaves stage, the trays were moved to a floating hydroponic system where treatments were applied. The NS contained N (140 mg/L, NO_3-N:NH_4-N 80:20), P (50 mg/L), Mg (40 mg/L), Ca (100 mg/L), and S (102 mg/L for K_{200} treatment and 52 mg/L for K_{50} and $K_{50\text{-}7d}$) and 2 levels of K, according to the treatment: 200 mg/L (K_{200}), a concentration usually used for growing baby leaf vegetables in a floating hydroponic system [6], and 50 mg/L (K_{50}), tested as a concentration to reduce K plant tissue concentration without detrimental effects on yield and quality. Independent NS tanks were used for each experimental unit. The following treatments were included in the experiment: K_{200} (K_{200} NS was used for the whole plant growing cycle), K_{50} (K_{50} NS was used for the whole plant growing cycle), and $K_{50\text{-}7d}$ (K_{200} NS was applied up to 7 days before harvest, and K_{50} NS was applied up to harvest). Rainwater was used to prepare the NS. Micronutrients were added as follows: B (0.27 mg/L), Mn (0.274 mg/L), Fe (0.22 mg/L), Zn (0.131 mg/L), Cu (0.032 mg/L), and Mo (0.0096 mg/L) [18]. The NS pH was adjusted to 5.5–6.0 using 1 M H_2SO_4, with negligible variations to the final concentration of S. A completely randomized design with 3 replications, each constituted by 576 plants available for sampling and analysis, was adopted for the study.

Table 1. Plant materials and dates of the experiment.

Species	Cultivar	Sowing	Treatment Application	Harvest I	Harvest II
Spinach (*Spinacia oleracea* L.)	Squirrel (Rjik Zwaan, De Lier, The Netherlands)	11/11/2016	22/11/2016	19/12/2016	04/01/2017
Swiss chard (*Beta vulgaris* L. ssp. *vulgaris*)	Rhubarb chard (Four Sementi, Piacenza, Italy)	19/12/2016	16/01/2017	15/02/2017	28/02/2017

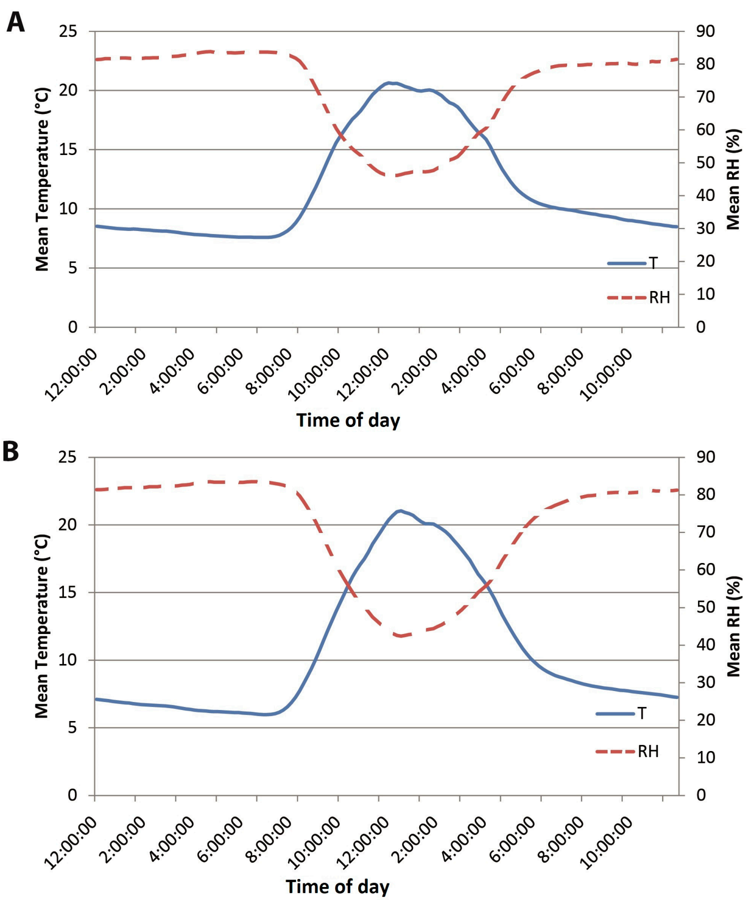

Figure 1. Average hourly temperature (T) and relative air humidity (RH) within the greenhouse during (**A**) spinach (11 Nov 2016 to 4 Jan 2017) and (**B**) Swiss chard (19 Dec 2016 to 28 Feb 2017) cultivation cycle.

2.2. Yield and Physical Measurements: Leaf Area, Color, and Dry Weight

At the harvest (4–5 true leaves stage), yield (expressed as fresh weight (FW) of shoots) and leaf area were determined on 25 randomly collected plants per replicate. Leaf area was measured with a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). Color parameters L* (lightness), a* (red), and b* (yellow) were measured on the peel surface of 10 leaves per replicate with a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in reflectance mode using the CIE L*a*b* color scale. Before the measurements, the colorimeter was calibrated with a standard reference with L*, a*, and b* values of 97.55, 1.32, and 1.41, respectively. Hue angle (h° = tan − 1(b*/a*)) and saturation or chroma (C = (a*2 + b*2)1/2) were then calculated from the primary readings. For the measurement of dry weight (DW), fresh leaf samples were maintained in a forced draft oven at 65 °C until constant weight was reached.

2.3. Extraction and Analysis of Ions

Ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector was performed as reported by D'Imperio et al. (2016) [3]. For determination of cation contents (Na, K, Mg, and Ca), 1 g of dried sample was ashed in a muffle furnace at 550 °C and digested with 20 mL of 1 M HCl in boiling water (99.5 ± 0.5 °C) for 30 min. The resulting solution was filtered, diluted, and analyzed by ion chromatography (Dionex DX120, Dionex Corporation) with a conductivity detector using an IonPac CG12A guard column and an IonPac CS12A analytical column (Dionex Corporation) at 35 °C, flow 1 mL/min. For determination of oxalate, 0.5 g DW samples were treated with 3.5 mM Na_2CO_3 and 1 mM $NaHCO_3$ for 30 min. After extraction, the samples were diluted and filtered using 0.45 µm RC followed by Dionex OnGuard IIP (Thermo Scientific) in order to remove organic compounds (phenolic fraction of humic acids, tannic acids, lignins, anthocyanins, and azo dyes from sample matrices). The resulting solutions were analyzed by ion chromatography (Dionex DX120, Dionex Corporation) with a conductivity detector, by using an IonPac AG14 precolumn and an IonPac AS14 separation column (Dionex Corporation) at 35 °C, flow 1 mL/min.

2.4. Extraction and Analysis of Total Polyphenol, Chlorophyll, and Carotenoid

The total polyphenol (TP) content in spinach and Swiss chard was determined by the Folin Ciocalteu method upon extraction by using the methods reported by Luthria et al. (2006) [19] with some modifications. Briefly, approximately 200 mg of lyophilized sample was mixed with 10 mL of the solvent mixture MeOH:H_2O:CH_3COOH (79:20:1 % v/v/v). The vials were then placed in a sonicator bath at ambient temperature for 30 min, followed by 1 h of magnetic stirring. The mixture was centrifuged at 10,000× *g*, 4 °C, for 10 min and the supernatant was transferred to a volumetric tube. The residue was resuspended in 10 mL of MeOH:H_2O:CH_3COOH (79:20:1 % v/v/v), gently mixed manually, and sonicated for an additional 30 min, followed by stirring (1 h) and centrifugation (10,000× *g*, 4 °C, 10 min). The supernatant was combined with the initial extract and appropriate aliquots of extracts were filtered and assayed for TP. For each sample, extractions and analyses were carried out in triplicate. The content of TP was determined using gallic acid (R^2 = 0.9921) as a calibration standard with a Perkin-Elmer Lambda 25 spectrophotometer (Perkin-Elmer, Boston, MA, USA).

Chlorophyll and carotenoid contents were determined spectrophotometrically using the extraction procedure reported by Montesano et al. (2018) [20]. Fresh samples were homogenized in 80% acetone, and the absorbance of the extract was measured at 662, 645, and 470 nm with a UV-1800 spectrophotometer (Perkin-Elmer Lambda 25, Boston, MA, USA).

2.5. Assessment of K, Mg, Ca, and Oxalate Bioaccessibility

Assessment of ion bioaccessibility (percentage of ions, K, Mg, Ca, and oxalate released from baby leaf vegetables during the in vitro gastrointestinal digestion process) was carried out as described by Ferruzzi et al. (2001) [21]. After the in vitro digestion process, the samples were centrifuged at 10,000× *g* for 1 h at 4 °C to separate the aqueous intestinal digesta (AQ) from the residual solid.

Aliquots of undigested AQ were collected, filtered using a 0.2 µm PTFE filter, and successively analyzed following the method used for vegetable materials. Blank correction was performed and subtracted in all analyses, in order to reset the contribution of blanks. With regard to Na bioaccessibility, the protocol applied [21] did not allow evaluation of Na bioaccessibility since blank correction was not performed for this cation. In fact, the Na released from the vegetable matrix during the digestion process was very low with respect to the Na present in the blank sample. This is probably related to reagents used in this protocol, as reported also by other authors [22]. The K, Mg, Ca, and oxalate contents in digested fluid were determined by the same protocol as that used for determination of ions in the vegetable material. Bioaccessibility was calculated as follows: (concentration in intestinal digesta/concentration food sample) × 100.

2.6. Statistical Analysis

Data were subjected to ANOVA using the general linear model procedure (Statistica 10.0, StatSoft, Tulsa, OK, USA) and means were separated by $LSD_{0.05}$ test with $p \leq 0.05$ considered to be statistically significant.

3. Results

3.1. Effect of Treatments on Yield, Leaf Area, Dry Matter, and Color Parameters

The effects of treatments on the growth and color parameters of plants were different according to the species under study. The reduction of K in the NS did not influence FW and leaf area in spinach, while in Swiss chard K_{50} and $K_{50\text{-}7d}$ reduced FW by 23% and 15%, respectively, and leaf area by 15.4%, on average, with respect to K_{200} control (Table 2). However, in both species dry matter was not influenced by K restriction (Table 2). In general, color was only slightly affected by treatments in spinach, with the exception of the C parameter, where the lowest value was observed in plants grown in low K conditions for the whole growing cycle (Table 2). On the contrary, clear effects on color were observed in Swiss chard as a result of K level in the NS. In particular, L*, a*, b*, and C showed higher values (in absolute terms) as an effect of K restriction, regardless of the duration of the low K conditions, with respect to control (43.1 vs. 41.57, −13.66 vs. −12.89, 25.58 vs. 23.91, and 29.01 vs. 27.17 on average, respectively), while no differences were observed in h° (Table 2).

Table 2. Effects of potassium (K) concentration in nutrient solution (NS) and duration of K restriction on yield (shoot fresh weight), leaf area, dry matter, and color parameters (L*, a*, b*, h°, C) of hydroponic spinach and Swiss chard.

Species	Treatment	Fresh Weight (g/m^2)	Leaf Area (cm^2/Plant)	Dry Matter (g/kg)	L*	A*	B*	h°	C
Spinach	K_{200}	2436	83.7	73.5	39.2	−15.6	24.9	122.1	29.4 ab
	K_{50}	2272	77.8	73.2	39.2	−15.4	24.3	122.4	28.7 b
	$K_{50\text{-}7d}$	2338	78.1	72.3	39.8	−15.7	25.4	121.8	29.9 a
	Significance	ns	ns	ns	ns	ns	ns	ns	*
	LSD	–	–	–	–	–	–	–	0.67
Swiss chard	K_{200}	2936 a	93.4 a	61.9	41.6 b	−12.9 b	23.91 b	118.3	27.2 b
	K_{50}	2260 c	77.3 b	67.4	43.0 a	−13.6 a	25.25 a	118.3	28.7 a
	$K_{50\text{-}7d}$	2488 b	80.7 b	63.4	43.2 a	−13.7 a	25.92 a	117.8	29.3 a
	Significance	***	***	ns	*	*	*	ns	**
	LSD	158	4.96	–	1.09	0.63	1.24	–	1.18

Means separation within columns by $LSD_{0.05}$. Significance: ns = not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. K_{200}: 200 mg/L K NS was used for the whole plant growing cycle; K_{50}: 50 mg/L K NS was used for the whole plant growing cycle; $K_{50\text{-}7d}$: K_{200} NS was applied up to 7 days before harvest, then K_{50} NS was applied up to harvest. LSD, least significant difference.

3.2. Effects of Treatments on Chlorophyll (a, b, and Total), Carotenoid, and Polyphenol Contents

The reduction of K in the NS did not significantly influence the chlorophyll content (a, b, and total). The mean values of CHLa, CHLb, and CHLtot were 639, 198, and 837 mg/g, respectively, in spinach, and 1157, 835, and 1993 mg/g FW, respectively, in Swiss chard. The restriction of K (K_{50} and $K_{50\text{-}7d}$) did not modify the carotenoid content in either species (143 and 198 mg/g FW on average, respectively, in spinach and Swiss chard; Table 3). Similarly, the TP content was not influenced by treatments in the present study regardless of the species, with mean values of 135 and 163 mg/100 g FW (Table 3).

Table 3. Effects of potassium (K) concentration in nutrient solution (NS) and duration of K restriction on chlorophyll (CHLa, CHLb, and CHLtot), carotenoid, and polyphenol content of hydroponic spinach and Swiss chard.

	Treatment	CHLa	CHLb	CHLtot	Carotenoid	Polyphenol
			(mg/g FW)			(mg/100 g FW)
Spinach	K_{200}	623	192	814	138	149
	K_{50}	673	209	881	155	121
	$K_{50\text{-}7d}$	622	195	816	136	137
	Significance	ns	ns	ns	ns	ns
Swiss chard	K_{200}	1031	696	1727	182	155
	K_{50}	1216	922	2139	191	184
	$K_{50\text{-}7d}$	1224	889	2113	220	150
	Significance	ns	ns	ns	ns	ns

Means separation within columns by $LSD_{0.05}$. Significance: ns = not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. K_{200}: 200 mg/L K NS was used for the whole plant growing cycle; K_{50}: 50 mg/L K NS was used for the whole plant growing cycle; $K_{50\text{-}7d}$: K_{200} NS was applied up to 7 days before harvest, then K_{50} NS was applied up to harvest. FW, fresh weight.

3.3. Potassium Content

In general, the K tissue content in spinach observed in this experiment was 5948 mg/kg FW on average (Table 4), and in Swiss chard was 3753 mg/kg FW on average. In spinach, the K_{50} treatment resulted in a K tissue concentration decrease of 26.9% with respect to K_{200} control, while reducing the K in the NS only during the last 7 days of the growth cycle ($K_{50\text{-}7d}$ treatment) resulted in a more moderate (7.3%) decrease in K tissue concentration (Table 4). On the other hand, in Swiss chard only the K_{50} treatment was able to induce a significant reduction of K tissue concentration compared to control (38.8%), while no significant differences were observed when the K concentration in the NS was reduced only during the last week of the growing cycle compared to normal conditions (Table 4).

Table 4. Effects of potassium (K) concentration in nutrient solution (NS) and duration of K restriction on Na, K, Mg, Ca, and oxalate content in edible parts of hydroponic spinach and Swiss chard.

	Treatment	K	Na	Mg	Ca	Oxalate
					(mg/kg FW)	
Spinach	K_{200}	6703 a	130 c	725	373	7150 a
	K_{50}	4899 c	470 a	889	557	5935 b
	$K_{50\text{-}7d}$	6244 b	267 b	769	398	7075 a
	Significance	***	*	ns	ns	**
	LSD	271	136	–	–	720
Swiss chard	K_{200}	4587 a	425 b	498 b	785 b	6566
	K_{50}	2805 b	615 a	706 a	1161 a	5616
	$K_{50\text{-}7d}$	3868 a	465 b	582 b	868 b	6792
	Significance	***	***	***	*	ns
	LSD	785	109	107	237	–

Means separation within columns by $LSD_{0.05}$. Significance: ns = not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. K_{200}: 200 mg/L K NS was used for the whole plant growing cycle; K_{50}: 50 mg/L K NS was used for the whole plant growing cycle; $K_{50\text{-}7d}$: K_{200} NS was applied up to 7 days before harvest, then K_{50} NS was applied up to harvest. FW, fresh weight.

3.4. Mg, Ca, and Oxalate Content

K_{50} treatment caused a 260% increase of Na content in spinach and 44% in Swiss chard, while $K_{50\text{-}7d}$ caused an increase of Na content (105%) only in spinach (Table 4). The application of K restriction did not influence the levels of Mg and Ca in spinach, whereas increases of 41% and 47%, respectively, were observed in Swiss chard plants grown in K_{50} NS compared to K_{200} control (Table 4). The restriction of K did not influence the content of oxalate in Swiss chard, while K_{50} treatment resulted in 17% lower oxalate content in spinach (Table 4).

3.5. Bioaccessibility of K, Mg, Ca, and Oxalate after In Vitro Digestion Process

The processes tested in this study, aimed at reducing the K content in edible parts of spinach and Swiss chard (K_{50} and $K_{50\text{-}7d}$), did not modify the K release in digested fluid with respect to control (K_{200}), regardless of the species. K bioaccessibility was, on average, 59.4% and 56.4% in spinach and Swiss chard, respectively (Table 5). As regards Mg and Ca, the tested process did not influence the release of these ions during the in vitro digestion process. Mg bioaccessibility was, on average, 54.6% and 52.9%, while Ca bioaccessibility was, on average, 32.1% and 10.3%, in spinach and Swiss chard, respectively (Table 5), despite the increase of Ca content observed in Swiss chard under K_{50} and $K_{50\text{-}7d}$ (Table 4). The K restriction process did not influence the bioaccessibility of oxalate (57% and 52.5%, on average, in spinach and Swiss chard, respectively; Table 5).

Table 5. Effects of potassium (K) concentration in nutrient solution (NS) and duration of K restriction on ion bioaccessibility (%) after in vitro gastrointestinal digestion process of hydroponic spinach and Swiss chard.

	Treatment	K	Mg	Ca	Oxalate
			(%)		
Spinach	K_{200}	54.9	54.6	36.7	52.1
	K_{50}	63.2	54.2	31.1	55.4
	$K_{50\text{-}7d}$	60.3	55.0	28.5	63.5
	Significance	ns	ns	ns	ns
Swiss chard	K_{200}	49.3	47.2	9.0	47.3
	K_{50}	62.1	58.7	12.4	52.3
	$K_{50\text{-}7d}$	57.9	53.0	9.6	57.8
	Significance	ns	ns	ns	ns

Means separation within columns by $LSD_{0.05}$. Significance: ns = not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. K_{200}: 200 mg/L K NS used for the whole plant growing cycle; K_{50}: 50 mg/L K NS was used for the whole plant growing cycle; $K_{50\text{-}7d}$: K_{200} NS was applied up to 7 days before harvest, then K_{50} NS was applied up to harvest.

4. Discussion

The present study reports the successful soil-less production of two baby leaf species (spinach and Swiss chard) with low K contents for CKD, also providing, for the first time, to the best of our knowledge, an evaluation of ion bioaccessibility after an in vitro gastrointestinal digestion process. We used an NS with overall ion composition similar to what was reported by Hoagland and Arnon [23], but we reduced the K concentration from 200 mg/L (usually used for growing baby leaf vegetables in a soilless system) to 50 mg/L. We found that in these growing conditions, K content in baby leaf vegetables was successfully reduced by about 39% and 27% in Swiss chard and spinach, respectively (Table 4). The K requirement for optimal plant growth is in the range of 2–5% of the dry weight of the plant's vegetative parts [24]. For spinach, although plants subjected to K deprivation showed a significant decrease in K tissue level, the K tissue concentrations were always in the sufficiency range (3.6% and 4.6%, respectively, in K_{50} and $K_{50\text{-}7d}$, considering average dry matter of 7.3%; Table 2). In fact, plants did not show any typical symptoms of K deficiency. On the other hand, Swiss chard plants subjected to K deprivation in the experimental conditions showed a K concentration lower than or close to the limit of sufficiency range (1.8 and 2.5%, respectively, in K_{50} and $K_{50\text{-}7d}$, considering average dry matter of 6.4% (Table 2)), as confirmed by the negative effects observed in growth parameters for this species (Table 2).

Our findings demonstrate that in the conditions of the study, reduced K concentration in the NS is effective for producing baby leaf vegetables with reduced K content for CKD patients. At the same time, the overall crop performance of spinach was not influenced by the K deprivation conditions tested in the study, in either quality or yield terms, while for Swiss chard a slight reduction of yield and a little modification of color parameters were observed (Table 2). The yield decrease in Swiss chard could be the result of the potentially detrimental effects of K deficiency in plant tissues on important physiological mechanisms in the plant, such as impairment of stomatal opening, thereby affecting CO_2 fixation [25]. Considering that the proposed cultivation technique is aimed at producing a niche product, i.e., food tailored for a restricted population (CKD patients), we consider a 15–23% yield reduction satisfactory if the product is compliant with the normal quality standard, as in our case. Anyway, considering that for CKD patients the K intake from food must be restricted to 1500 mg per day [26], it is important to note that 100 g of baby Swiss chard grown using NS with a low K level (50 mg/L) would provide about 19% of the recommended K daily intake, while 100 g of the same baby leaf vegetable grown with usual K concentration (200 mg/L) would provide about 31% of the recommended intake. Similarly, 100 g of baby spinach would provide about 33% and 45% of the K daily intake recommended for CKD patients in the case of low and usual K concentration in the NS, respectively.

Dietetic-nutritional therapy is an important component of the conservative treatment of patients suffering from CKD that must anticipate and be integrated with pharmacological therapy [27]. The current nutritional approach is to limit the consumption of food sources rich in K, including vegetables, with the aim of reducing the intake of this nutrient. However, a diet low in vegetables and fruits also results in a reduction of vitamins, minerals, and bioactive compounds, generally with antioxidant and anti-inflammatory activity, as well as alteration of the intestinal microbiota [28–30]. In advanced stages of CKD, a state of dysbiosis of the intestinal microbiota occurs, with alteration of intestinal permeability and bacterial composition, imbalance of microbial metabolism in the proteolytic sense, and increased production of uremic toxins, such as p-cresol and indoxyl sulfate [29]. The results of the present study suggest that the availability of baby leaf vegetables with reduced K content could allow reducing K intake for the same serving of vegetables and/or increasing the amount of servings without excessively increasing K intake. Our findings, in agreement with other studies focused on the reduction of K tissue content in leafy vegetables [16,17], suggest that the effect is species-dependent. This underlines the opportunity to select appropriately targeted genotypes suitable for cultivation processes in order to produce food products tailored for specific nutritional needs, such as those of CKD patients.

When the K content in the NS was reduced to 50 mg/L, the average Na content increased in both baby leaf species (Table 4). We would like to point out that rainwater was used in the experiment and no Na was intentionally added in the NS preparation. The concentration of Na in the final NS was negligible (≈8 mg/L), as an effect of impurities normally present in fertilizers and stored rainwater. For Swiss chard, increased Ca and Mg was observed (Table 4). According to Marchner [24], it is likely that plants compensate for K reduction by increasing the tissue concentration of cations with similar roles in physiological processes, such as enzyme activity, pH control, and osmotic regulation. In particular, the role of Na in replacing the K in both biochemical and physiological nonspecific functions should be considered [31]. From a nutritional point of view, it is important to highlight that high intake of Na may increase the risk of some diseases, thus the World Health Organization [32] recommends not exceeding a daily intake of 2000 mg. The results of the present study show that 100 g of baby leaf vegetables with reduced K content supplied 47 and 61 mg of Na from spinach and Swiss chard, respectively (Table 4). These amounts represent only 2–3% of the recommended daily intake and can be considered absolutely negligible with respect to the recommended limits.

Furthermore, according to the USDA National Nutrient Database for Standard Reference, values of Na concentration for spinach and Swiss chard are, respectively, 790 [11] and 2130 [12] mg/kg fresh weight, much higher than the values found in the present study. Furthermore, increased Mg and Ca content in Swiss chard represents an interesting result, considering that generally CKD is a complex disease and its progression is associated with a number of serious complications, including metabolic bone diseases [33]. In fact, preservation of bone is the primary focus of Ca control in kidney disease. Kidney failure reduces the production and conversion of vitamin D to active calcitriol 1.2(OH_2)D_3 that in normal kidney function controls the absorption of Ca in the intestinal tract during digestion. The therapeutic approach for CKD includes, in most cases, pharmacological treatment based on Ca supplementation [34]. The increased Ca and Mg, both cations associated with beneficial effects on bone mineral density, observed in the K_{50} Swiss chard may help to reduce the consumption of mineral supplements. Moreover, the potential availability of vegetables tailored for CKD patients could remedy the limitations these patients are generally subjected to in terms of consuming this food group, with the possibility of taking in healthy compounds typical of vegetables.

Regarding other nutritional traits, the K level in the NS did not affect the carotenoid, chlorophyll, or phenol content (Table 3), suggesting that by using NS with low K concentration, it is possible to obtain reduced K in baby leaves without negatively affecting important aspects of the vegetables' nutritional quality. Furthermore, we found that the oxalate content in spinach was successfully reduced by about 17% in samples grown with 50 mg/L of K with respect to spinach grown with 200 mg/L of K. The use of Chenopodiaceae with a high oxalate content as food may be associated with a negative impact on human health [35], due to the negative effects of this antinutritional compound on reduced bioavailability of Ca, Mg, and Fe in the intestinal tract during digestion [35,36]. Therefore, we can underline the positive impact of reduced K concentration in NS for baby leaf vegetable production with regard to reduced antinutritional compounds such as oxalate.

In addition to what was reported in other studies aimed at producing vegetables with low K content [16,17], we assessed the quality of K-reduced vegetables by evaluating ion bioaccessibility after in vitro gastrointestinal digestion. We found that the processes tested in this study for reducing K content in the edible parts of baby leaves did not modify the ion bioaccessibility in either species. Therefore, considering average K bioaccessibility of about 56.4% for Swiss chard (Table 5), hypothetical consumption of 100 g of baby leaf implies K bioaccessibility of 158 and 259 mg for K_{50} and K_{200} samples, respectively. At the same time, considering average K bioaccessibility of about 59.5% for spinach (Table 5), hypothetical consumption of 100 g of spinach would imply K bioaccessibility of 291 and 399 mg for K_{50} and K_{200} samples, respectively. Besides K, it would be interesting to evaluate the bioaccessibility of other ions. Thus, while Mg bioaccessibility appears to be similar in both species, Swiss chard showed Ca bioaccessibility about threefold lower than spinach. Also, compared to Ca bioaccessibility values observed in a previous study carried out by this research group on four leafy vegetable species (mizuna, tatsoi, basil, and endive), Swiss chard showed, on average, lower values [3]. At the same time, Swiss chard showed slightly lower bioaccessibility of oxalate. According to previous studies [2–5] the bioaccessibility of mineral nutrients can be considerably affected by several factors, including mineral type and food matrix composition. At any rate, all the results of the present study suggest that bioaccessibility, defined as the ability of a nutrient to be released into the gastrointestinal tract, can be considered as a useful tool to better estimate real nutrient intake from vegetable products, especially when innovative cultivation protocols are applied.

5. Conclusions

In general, we found a significant reduction in the K tissue content in baby leaf vegetables as a result of reducing the K concentration in the NS. The crop performance and quality traits as well as the bioaccessibility of ions were not affected at all in spinach, while a slight decrease in yield was observed in Swiss chard. The reduced-potassium spinach and Swiss chard obtained in this study might be proposed for CKD patients with the aim of reducing their daily K intake. At the same time, consumption of these leaf vegetables by CKD patients could allow an increase in the amount of servings, in order to take advantage of vitamins, minerals, and bioactive compounds with positive effects on health without excessively increasing K intake. Moreover, our key finding is that evaluation of ion bioaccessibility after in vitro gastrointestinal digestion could be considered a useful tool to better estimate the real nutrient intake from vegetable products, especially when innovative cultivation protocols are applied. Therefore, the results of the present study suggest that hydroponic production of reduced-potassium leaf vegetables can be considered as a tailored nutritional strategy for CKD patients, taking into consideration the easy applicability of the proposed cultivation technique compared with common production practices.

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References

1. Signore, A.; Renna, M.; D'Imperio, M.; Serio, F.; Santamaria, P. Preliminary evidences of biofortification with iodine of "Carota di Polignano", an italian carrot landrace. *Front. Plant Sci.* **2018**, *9*, 170. [CrossRef] [PubMed]
2. D'Imperio, M.; Renna, M.; Cardinali, A.; Buttaro, D.; Santamaria, P.; Serio, F. Silicon biofortification of leafy vegetables and its bioaccessibility in the edible parts. *J. Sci. Food Agric.* **2016**, *96*, 751–756. [CrossRef] [PubMed]
3. D'Imperio, M.; Renna, M.; Cardinali, A.; Buttaro, D.; Serio, F.; Santamaria, P. Calcium biofortification and bioaccessibility in soilless "baby leaf" vegetable production. *Food Chem.* **2016**, *213*, 149–156. [CrossRef] [PubMed]
4. D'Imperio, M.; Montesano, F.F.; Renna, M.; Leoni, B.; Buttaro, D.; Parente, A.; Serio, F. NaCl stress enhances silicon tissue enrichment of hydroponic "baby leaf" chicory under biofortification process. *Sci. Hort.* **2018**, *235*, 258–263. [CrossRef]
5. Montesano, F.F.; D'Imperio, M.; Parente, A.; Cardinali, A.; Renna, M.; Serio, F. Green bean biofortification for Si through soilless cultivation: Plant response and Si bioaccessibility in pods. *Sci. Rep.* **2016**, *6*, 31662. [CrossRef] [PubMed]
6. Golubkina, N.; Kekina, H.; Caruso, G. Yield, quality and antioxidant properties of Indian mustard (*Brassica juncea* L.) in response to foliar biofortification with selenium and iodine. *Plants* **2018**, *7*, 80. [CrossRef] [PubMed]
7. Golubkina, N.; Zamana, S.; Seredin, T.; Poluboyarinov, P.; Sokolov, S.; Baranova, H.; Krivenkov, L.; Pietrantonio, L.; Caruso, G. Effect of selenium biofortification and beneficial microorganism inoculation on yield, quality and antioxidant properties of shallot bulbs. *Plants* **2019**, *8*, 102. [CrossRef] [PubMed]
8. Mleczek, M.; Siwulski, M.; Rzymski, P.; Budzyńska, S.; Gąsecka, M.; Kalač, P.; Niedzielski, P. Cultivation of mushrooms for production of food biofortified with lithium. *Eur. Food Res. Technol.* **2017**, *243*, 1097–1104. [CrossRef]
9. Tonelli, M.; Wiebe, N.; Culleton, B.; House, A.; Rabbat, C.; Fok, M.; Garg, A.X. Chronic kidney disease and mortality risk: A systematic review. *J. Am. Soc. Nephrol.* **2006**, *17*, 2034–2047. [CrossRef] [PubMed]
10. World Kidney Day: Chronic Kidney Disease (2015). Available online: http://www.worldkidneyday.org/faqs/chronic-kidney-disease/ (accessed on 4 March 2019).
11. U.S. Department of Agriculture (USDA) Database. Available online: https://ndb.nal.usda.gov/ndb/foods/show/11457?fgcd=&manu=&format=&offset=&sort=default&order=asc&ds=&qt=&qp=&qa=&qn=&q=&ing (accessed on 11 September 2019).

12. U.S. Department of Agriculture (USDA) Database. Available online: https://ndb.nal.usda.gov/ndb/foods/show/11147?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=11147+Swiss+chard&ds=&qt=&qp=&qa=&qn=&q=&ing= (accessed on 11 September 2019).
13. Shin, R. Strategies for improving potassium use efficiency in plants. *Mol. Cell* **2014**, *37*, 575–584. [CrossRef] [PubMed]
14. Bergmann, W. Causes, development and diagnosis of symptoms resulting from mineral. Macronutrients: Potassium. In *Nutritional Disorders of Plants: Development, Visual and Analytical Diagnosis*; Bergmann, W., Fischer, G., Eds.; Gustav Fisher Verlag: Jena, Germany, 1992; pp. 117–132.
15. Ruiz, J.M.; Romero, L. Relationship between potassium fertilisation and nitrate assimilation in leaves and fruits of cucumber (*Cucumis sativus*) plants. *Ann. Appl. Biol.* **2002**, *140*, 241–245. [CrossRef]
16. Renna, M.; Castellino, M.; Leoni, B.; Paradiso, V.M.; Santamaria, P. Microgreens production with low potassium content for patients with impaired kidney function. *Nutrients* **2018**, *10*, 675. [CrossRef] [PubMed]
17. Ogawa, A.; Eguchi, T.; Toyofuku, K. Cultivation methods for leafy vegetables and tomatoes with low potassium content for dialysis patients. *Environ. Control Biol.* **2012**, *50*, 407–414. [CrossRef]
18. Johnson, C.M.; Stout, P.R.; Broyer, T.C.; Carlton, A.B. Comparative chlorine requirements of different plant species. *Plant Soil* **1957**, *8*, 337–353. [CrossRef]
19. Luthria, D.L.; Mukhopadhyay, S.; Krizek, D.T. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *J. Food Compos. Anal.* **2006**, *19*, 771–777. [CrossRef]
20. Montesano, F.F.; van Iersel, M.W.; Boari, F.; Cantore, V.; D'Amato, G.; Parente, A. Sensor-based irrigation management of soilless basil using a new smart irrigation system: Effects of set-point on plant physiological responses and crop performance. *Agric. Water Manag.* **2018**, *203*, 20–29. [CrossRef]
21. Ferruzzi, M.G.; Failla, M.L.; Schwartz, S.J. Assessment of degradation and intestinal cell uptake of carotenoids and chlorophyll derivatives from spinach puree using an in vitro digestion and Caco-2 human cell model. *J. Agric. Food Chem.* **2001**, *49*, 2082–2089. [CrossRef]
22. Hamilton, E.M.; Barlow, T.S.; Gowing, C.J.B.; Watts, M.J. Bioaccessibility performance data for fifty-seven elements in guidance materials BGS 102. *Microchem. J.* **2015**, *123*, 131–138. [CrossRef]
23. Hoagland, D.R.; Arnon, D.I. The water-culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Stn.* **1950**, *347*, 1–32.
24. Marschner, H. *Mineral Nutrition of Higher Plants*; Academic Press: Cambridge, MA, USA, 1995; ISBN 9780080571874.
25. Cakmak, I. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J. Plant Nutr. Soil Sci.* **2005**, *168*, 521–530. [CrossRef]
26. Putcha, N.; Allon, M. Management of hyperkalemia in dialysis patients. *Semin. Dial.* **2007**, *20*, 431–439. [CrossRef] [PubMed]
27. Cupisti, A.; Brunori, G.; Di Iorio, B.R.; D'Alessandro, C.; Pasticci, F.; Cosola, C.; Bellizzi, V.; Bolasco, P.; Capitanini, A.; Fantuzzi, A.L.; et al. Nutritional treatment of advanced CKD: Twenty consensus statements. *J. Nephrol.* **2018**, *31*, 457–473. [CrossRef] [PubMed]
28. Vaziri, N.D.; Wong, J.; Pahl, M.; Piceno, Y.M.; Yuan, J.; De Santis, T.Z.; Andersen, G.L. Chronic kidney disease alters intestinal microbial flora. *Kidney Int.* **2013**, *83*, 308–315. [CrossRef] [PubMed]
29. Montemurno, E.; Cosola, C.; Dalfino, G.; Daidone, G.; De Angelis, M.; Gobbetti, M.; Gesualdo, L. What would you like to eat, Mr CKD microbiota? A Mediterranean diet, please! *Kidney Blood Pres. Res.* **2014**, *39*, 114–123. [CrossRef] [PubMed]
30. Vera, M.; Torramade-Moix, S.; Martin-Rodriguez, S.; Cases, A.; Cruzado, J.M.; Rivera, J.; Diaz-Ricart, M. Antioxidant and Anti-Inflammatory Strategies Based on the Potentiation of Glutathione Peroxidase Activity Prevent Endothelial Dysfunction in Chronic Kidney Disease. *Cell. Physiol. Biochem.* **2018**, *51*, 1287–1300. [CrossRef] [PubMed]
31. Flowers, T.J.; Lauchli, A. Sodium versus potassium: Substitution and compartmentation. *Encycl. Plant Physiol.* **1983**, *15*, 651–681.
32. World Health Organization. Available online: https://www.who.int/mediacentre/news/notes/2013/salt_potassium_20130131/en/ (accessed on 2 March 2019).
33. Thomas, R.; Kanso, A.; Sedor, J.R. Chronic kidney disease and its complications. *Prim. Care* **2008**, *35*, 329–344. [CrossRef] [PubMed]

34. Cannata-Andía, J.B.; Minerva Rodriguez, G.; Carlos Gómez, A. Osteoporosis and adynamic bone in chronic kidney disease. *J. Nephrol.* **2013**, *26*, 73–80.
35. Gupta, S.; Lakshmi, J.A.; Prakash, J. In vitro bioavailability of calcium and iron from selected green leafy vegetables. *J. Sci. Food Agric.* **2006**, *86*, 2147–2152. [CrossRef]
36. Siener, R.; Honow, R.M.; Seidler, A.; Voss, S.; Hesse, A. Oxalate contents of species of the Polygonaceae, Amaranthaceae and Chenopodiaceae families. *Food Chem.* **2006**, *98*, 220–224. [CrossRef]