Study on cultivated low-potassium lettuce (*Lactuca sativa* L.) in plant factory

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1. Abstract

Patients with end stage renal disease (ESRD) often experience hyperkalemia, a condition that requires limiting their dietary intake of potassium. Fresh vegetables are the largest source of potassium intake for Taiwanese people, it is vital to provide vegetables with low potassium concentration for ESRD patents. Prior study was conducted in Japan for low-potassium vegetable production, however, some products are low in potassium but high in sodium. The objective of this study was to develop an operating procedure enable to grow low potassium, low sodium and low nitrate lettuces without scarifying too much of the fresh weight. The frill ice lettuces were grown in a plant factory with artificial lighting (PFAL) using hydroponic system with deep flow technique (DFT). The lettuces were grown at environment with average light intensity of 200 μmol $m^{-2}s^{-1}$, light period of 16 hours, day/night temperatures of 25/20°C and carbon dioxide concentration of 1,200 ppm. The Yamasaki solution was used, termed 100% potassium as the control group and the growing period from sowing to harvest was 42 days. For the treatment group, potassium ions were removed during the treatment period (measured in weeks) varied prior to harvest. Two procedures were developed for growing frill ice lettuce. The 1st can reduced potassium content by approximately 90%, however, the fresh weight also reduced by 48%. The 2nd can reduced potassium content by 57.7 % and reduced fresh weight by only 4.7%. This study shows the potential of producing low potassium (< 90), low sodium (< 25) and low nitrate (< 2500) frill-ice lettuce in a PFAL.

Keywords: End Stage Renal Disease, Hyperkalemia, Hydroponics, Nitrate, Sodium, Potassium.

2. INTRODUCTION

End Stage Renal Disease (ESRD) patients were often required to undergo alternative treatments such as hemodialysis, peritoneal dialysis, and kidney transplant. ESRD incidence and prevalence rates have increased with economic development. According to a 2016 report on ESRDS situation of 2014, that in regions that had relatively more rapid economic development such as those in Europe, the U.S.A., and Japan are seeing growing ESRD prevalence rates. At 3,219 per million people^{-1}, Taiwan currently has the world's highest ESRD prevalence rate, whereas Japan and the U.S.A. have an ESRD prevalence rate of 2,505 and 2,076 per million people, respectively. At 455 per million people^{-1}, Taiwan currently has the world's highest ESRD incidence rate, whereas Japan and the U.S.A. have an ESRD incidence rate of 285 and 370 per million people, respectively. According to Taiwan's National Health Insurance Database, as of July 2014, patients with chronic renal failure or uremia and who must regularly receive dialysis treatment totaled 75,683, indicating that renal disease has become a critical disease over the years.

Nutritional care for patients with chronic kidney disease (CKD) differs between those who are at different disease stages. In addition, attention must be paid to their dietary intake of minerals, in which potassium ions serve as a crucial indicator. For patients with stage 1 to stage 4 renal failure, their permissible potassium intake is determined by their blood potassium test results. For patients with stage 5 renal failure who are also receiving hemodialysis, they may consume 2000 ~ 3000 mg of potassium per day, whereas those who are also receiving peritoneal dialysis may consume 3000 ~ 4000 mg of potassium per day (Beto & Bansal, 2004 (Beto and Bansal, 2004); Tritt, 2004). Patients who consume an excessively high potassium ion content will likely experience hyperkalemia, which leads to cardiac arrhythmia. Therefore, patients with kidney disease must reduce their intake of vegetables and fruits containing high potassium. Taiwan's Food and Drug Administration defines high potassium vegetables as those that contain more than 300 mg of potassium per 100 g of the vegetables. By blanching and stir frying, potassium content of the vegetables will be reduced, however, many vegetables such as lettuce are raw food.

Plant factory with artificial lighting (PFAL) enables precise control of the environment, eliminate dependence on the natural environment, and allow planned crop productions. In addition, PFAL can produce healthy, safe, and high-quality crops; the secondary metabolites of these crops may also be regulated (Lee et al., 2014). Furthermore, PFAL enable developing edible vegetables for patients as well as adjusting nutrient content according to patients' needs. Because fresh vegetables are the largest source of potassium intake (approximately 30%; Wu et al., 2011) for Taiwanese people, by lowering the potassium content of the vegetables, patients who are required to watch their potassium intake will be provided with more dietary options.CKD patients with high incidence of hypertension. In 2003, 86% of US dialysis patients had hypertension (Agarwal, et al., 2003). Hypertensive patients for sodium intake is limited. Previous studies have shown that low-potassium lettuce can significantly increase the sodium ions and nitrate content decreased (Yoshida, et al., 2014). According to EU regulations, the nitrate content in lettuce raw food must be less than 2500 ppm, in order to avoid risks to human health. In this study, frill ice lettuces were the target crop and their potassium ion content was reduced and maintaining the low sodium and low nitrate content to produce an edible raw vegetable that can be consumed by patients with kidney disease.

3. MATERIALS AND METHODS

Experiment materials

Experiment 1

Frill ice lettuces (*Lactuca sativa* L.), the target crop, were grown in a controlled environment of a PFAL for 6 weeks with the controlled carbon dioxide content of 1200 ± 100 ppm, the light source used was cool white, 6,500 K LED tubes and the PPFD was kept at 200 μmol m^{-2} s^{-1}.

The production was divided into 3 stages: the seedling, the growing, and the treatment stages. Table 1 shows the operating codes of the treatment groups during the last 3 weeks prior to harvest. The descriptions of operating codes and the operating codes of the first 2 stages can be found in the note of Table 1.

The cultural solution with known formula was administered during all six weeks for the control group (6K), and a nutrient solution contained 0% potassium (replacing K^+ with NH_4^+) was administered during the started from the 4th, 5th, 6th week for the group with treatment code of 5K, 4K, and 3K, respectively. Samples were collected on DAS 28, 35, and 42 to measure the fresh weight of the plants and the mineral contents of the plants and the cultural solutions.

Table 1 Operating codes during the treatment stages for low potassium lettuce production in experiment 1.

<table>
<tr><th>Treatment code</th><th>Treatment Stage 1
DAS 21-28</th><th>Treatment Stage 2
DAS 28-35</th><th>Treatment Stage 3
DAS 35-42</th></tr>
<tr><td>6K (CK)</td><td rowspan="3">N1_E1.2_L200_d26_H16_A25/20</td><td colspan="2">Same as Treatment Stage 1</td></tr>
<tr><td>5K</td><td>Same as Treatment Stage 1</td><td>N2_E1.2_L200_d26_H16_A25/18</td></tr>
<tr><td>4K</td><td colspan="2">N2_E1.2_L200_d26_H16_ A25/18</td></tr>
<tr><td>3K</td><td colspan="3">N2_E1.2_L200_d26_H16_A25/20</td></tr>
</table>

Note: DAS: Days After Sowing

Seedling stage (DAS: 0-7): N1_E1.2_L200_d910_H24_A25

Growing stage (DAS: 7-21): N1_E1.2_L200_d45_H16_A25/20

Operating code:

Nx: N, Nutrient solutions.

N1: Regular Nutrient solutions (100% potassium)

N2: Potassium-free Nutrient solution (0% potassium)

E_x: E, Electrical conductivity (EC) of nutrient solution,

x, Value of EC, Unit: mS·cm^{-1}.

L_x: L, LED tube (cool white, color temperature 6500 K)

x : Value of light intensity, Unit : μmol·m^{-2}·s^{-1}.

dx: d, Cropping density,

x, Value of the cropping density, Unit : plts·m^{-2}

H_x: H, Duration of light period,

x : Hours of light period per day, Unit :˙ hours day^{-1}

AdT/nT: A, average day/night temperature, Unit :℃

Experiment 2

Frill ice lettuces (*Lactuca sativa* L.), the target crop, were grown in a controlled environment of a PFAL for 6 weeks with the controlled carbon dioxide content of 1200 ± 100 ppm, the light source used was cool white, 6,500 K LED tubes and the PPFD was kept at 200 μmol m^{-2} s^{-1}.

The production was divided into 3 stages: the seedling, the growing, and the treatment stages. Table 2 shows the operating codes of the treatment groups during the last 2 weeks prior to harvest. The descriptions of operating codes and the operating codes of the first 2 stages can be found in the note of Table 2.

The cultural solution with known formula was administered during the first 4 weeks for all treatments and the last 2 weeks for the control group (coded 2.0 K), Other 5 treatments in the 5th and 6th week were provided partial strength of the potassium coded 1.5K (75% K, 75% K for week 5 and 6, respectively), 1.25K (75% K + 50% K), 1.00K (50% K + 50% K), 0.75K (50% K + 25% K) and 0.50K (0.25 K + 0.25 K). Samples were collected on DAS 42 to measure the fresh weight of the plants and the mineral contents of the plants and the cultural solutions.

Table 2 Operating codes during the treatment stages for low potassium lettuce production in experiment 2.

Treatments	Treatment Stage 1 (DAS 28-35)	Treatment Stage 2 (DAS 35-42)
2.0K (CK)	N1_E1.2_L200_d26_H16_A25/20	
1.5K	N3_E1.2_L200_d26_H16_A25/20	
1.25K	N3_E1.2_L200_d26_H16_A25/20	N4_E1.2_L200_d26_H16_A25/20
1.00K	N4_E1.2_L200_d26_H16_A25/20	
0.75K	N4_E1.2_L200_d26_H16_A25/20	N5_E1.2_L200_d26_H16_A25/20
0.50K	N5_E1.2_L200_d26_H16_A25/20	

Note: DAS: Days After Sowing

Seedling stage (DAS 0-7): N1_E1.2_L200_d910_H24_A25

Growing stage (DAS: 7-28): N1_E1.2_L200_d45_H16_A25/20

Operating code:

Nx: N, Nutrient solutions

N1: Regular Nutrient solution (100% potassium)

N3: 75% Potassium Nutrient solution
N4: 50% Potassium Nutrient solution
N5: 25% Potassium Nutrient solution.

E_x: E, Electrical conductivity (EC) of nutrient solution,
x, Value of EC, Unit: $mS \cdot cm^{-1}$.

L_x: L, LED tube (cool white, color temperature 6500 K)
x : Value of light intensity, Unit : $\mu mol \cdot m^{-2} \cdot s^{-1}$.

dx: d, Cropping density,
x, Value of cropping density, Unit : $plts \cdot m^{-2}$

H_x: H, Duration of light period,
x : Hours of light period per day, Unit : $hours\ day^{-1}$

A dT/nT: A, average day/night temperature, Unit :℃.

Analytical method

Fresh weight

The fresh weight of shoots and roots of lettuce was measured using an electronic balance.

The mineral content of plants

Plants to take complete leaves, take 1g, frozen at -20 ° C for 48 hours grinding extraction dilution to the appropriate concentration (He et al., 1998). The samples were analyzed for their anion and cation contents using an ion analyzer (IA-300, DKK-TOA Corporation, Japan, Cationic column:PCI-205l, Anion column:PCI-322).

Statistical analysis

The results were analyzed using Duncan's multivariate analysis using statistical software SAS 9.1. Differences were considered significant when $p < 0.05$.

4. RESULTS AND DISCUSSION

As shown in Fig.1, label 6K means lettuce was grown under 6 weeks of regular nutrient solution with full strength potassium and 4K means 2 weeks prior to harvest plants were grown in potassium-free solution. Dramatic decrease was found in the fresh weight of the upper part of the plant but not the root, as shown in Table 2. With the increase of treatment time, not only the fresh weight of the upper part, but also the potassium content and the nitrate content of the lettuce decreased (Fig. 2, Fig. 3 and last column of Table 3).

Potassium is one of the essential elements in plant growth, it influences enzyme activities, pore opening and closing, photosynthesis, protein synthesis, and crop quality (Pettigrew, 2008). Potassium exists in plant bodies in the form of ions, which make it highly mobile. When plants contain insufficient amount of potassium, old leaves turn yellow and plant growth is negatively affected. In this study, the lettuces treated using potassium-free nutrient solution for one week showed a significant reduction in their fresh weight; this finding supports those obtained in previous studies.

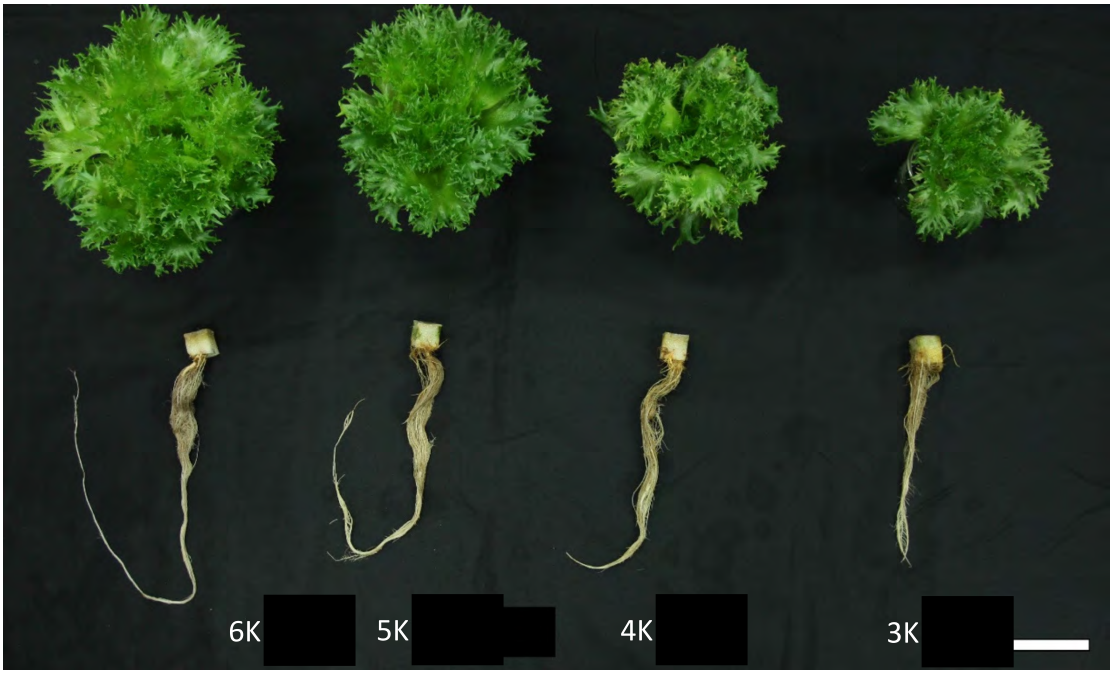

Fig. 1 Effects of three week treatments (DAS 21~42) with potassium-free solution on the growth of frill-ice lettuce harvest on DAS 42 (Label 6K means 6 weeks of regular nutrient solution with full strength on potassium (100% K), 4K means 4 weeks with 100% K and 2 weeks prior to harvest with potassium-free (0% K) solution). Bar=10cm.

Table 2 Effect of three week treatments (DAS 21~42) of potassium-free nutrient solution on the fresh weight of frill ice lettuce harvested on DAS 42

Treatments	Shoot Fresh Weight g plt^{-1}	Root Fresh Weight g plt^{-1}	Root/Shoot
6K	108.67 a	11.16 a	0.10 c
5K	87.40 b	10.76 a	0.12 c
4K	56.20 c	9.35 a	0.17 b
3K	32.30 d	9.54 a	0.30 a

Means followed by the different letters in each column are significantly different at 5% level by Duncan's Multiple Range Test.

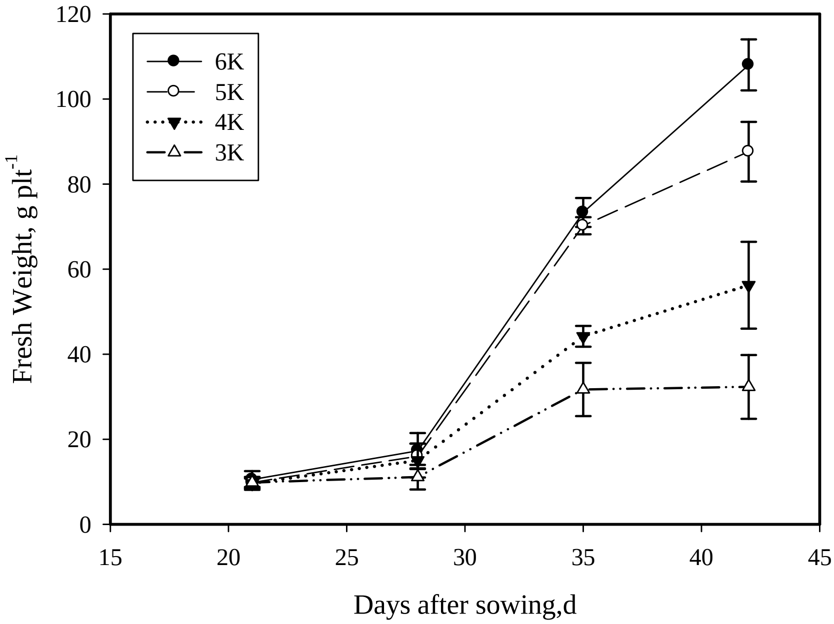

Fig. 2 Effect of three week treatments (DAS 21~42) of potassium-free nutrient solution on the fresh weight of frill-ice lettuce

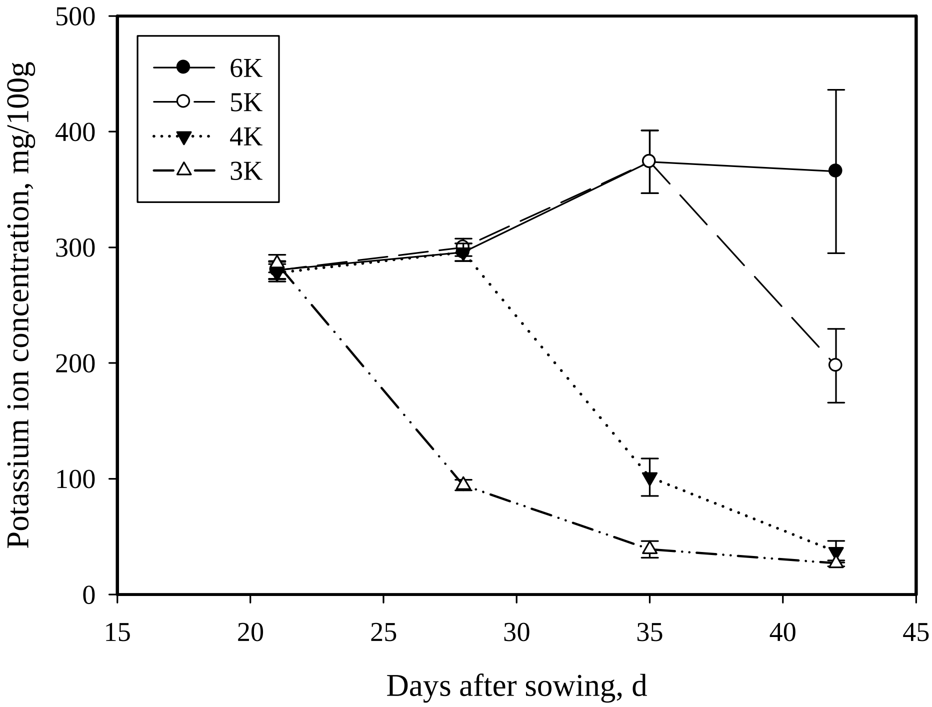

Fig. 3 Effect of three week treatments (DAS 21~42) of potassium-free nutrient solution on the potassium ion concentration of frill-ice lettuce

Regarding mineral contents of the frill-ice lettuce, Table 3 shows significant increase of the sodium and magnesium ions and significant decreased of the potassium and nitrate ions as the treatment time for the potassium-free nutrient solution increased. Potassium ion is critical cation used by guard cells to control pore opening and closing in plants. Plants deprived of potassium are likely to experience closed pores (Marschner, 1995), which results in the accumulation of reactive oxygen species (ROS) and the generation of malondialdehyde (MDA), the latter of which impedes plant growth. Low concentration of sodium ions can replace potassium ions and be introduced to guard cells to open pores (Lv et al., 2012). In an environment with a potassium–sodium ratio of 0.03/2.97 mM, beets (*Beta vulgaris* L.) showed a MDA level significantly lower than that treated with a potassium-deficient nutrient solution. In addition, studies have shown that the absorption of sodium ions increased plants' osmoregulation, whereas a reduction in free amino acid content allowed plants to maintain more favorable growth in a potassium-deficient environment (Pi et al., 2014). The present study found that lettuces grown using a potassium-deficient nutrient solution showed high sodium ion content in their stems, which may have been caused by the lettuces actively absorbing sodium ions to maintain growth. Although the lettuces contained a high sodium ion content, the sodium ion content of the 4K treatment was no more than the average sodium content of locally grown lettuces at approximately 24 mg per 100 g.

Table3 Effects of three week treatments (DAS 21~42) of potassium-free nutrient solution on the mineral contents of frill-ice lettuce

Treatments	Na (mg/100g)	K (mg/100g)	Mg (ppm)	Ca (ppm)	NO_3 (ppm)
6K	2.53 c	365.52 a	80.96 b	194.80 a	6157.60 a
5K	4.31 c	197.68 b	95.84 b	86.72 c	4150.48 b
4K	22.23 b	36.96 c	109.20 b	66.32 c	609.26 c
3K	42.23 a	26.90 c	217.76 a	148.61 b	388.03 c

Means followed by the different letters in each column are significantly different at 5% level by Duncan's Multiple Range Test.

Results of experiment 2 show that various two week treatments have no significant effect on fresh weight (Fig. 4) and the contents of the sodium and calcium ions of the upper part of lettuce (Table 4). Results shown in Fig. 4 and Table 4 also indicate that the 0.75 K treatment can significantly reduce the potassium and nitrate contents of lettuce by 57% and 52% without scarifying the fresh weight.

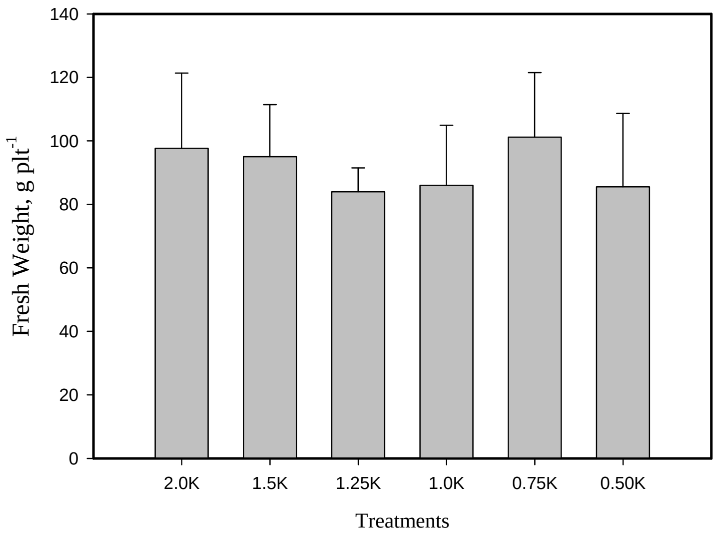

Fig. 4 Effect of two week treatments (DAS 28~42) of potassium-deficient nutrient solution on the fresh weight of frill-ice lettuce.

Table 4 Effects of two week treatments (DAS 28~42) of potassium-deficient nutrient solution on the mineral contents of frill-ice lettuce

Treatments	Na (mg/100g)	K (mg/100g)	Mg (ppm)	Ca (ppm)	NO_3 (ppm)
2.00K	3.19 a	408.05 a	220.20 a	327.68 a	3085.0 a
1.50K	3.27 a	318.34 a	133.67 b	308.00 a	2546.2 b
1.25K	2.53 a	212.50 b	126.47 b	270.13 a	1480.0 c
1.00K	3.02 a	242.30 b	163.44 b	270.96 a	2446.4 b
0.75K	2.44 a	172.53 c	155.76 b	216.72 a	1493.6 c
0.50 K	3.22 a	188.53 c	180.73 b	304.08 a	2278.5 b

Means followed by the different letters in each column are significantly different at 5% level by Duncan's Multiple Range Test.

Patients with CKD often experience hyperkalemia, nutrition-based treatments are a crucial clinical method used to treat such patients (Saxena, 2012). The average daily dietary potassium intake of Taiwanese elderly male adults (i.e., more than 65 years of age) was reported to be 2,798 mg, approximately 30% of which come from vegetables (Wu et al., 2011). For patients with CKD, their recommended daily potassium intake is 1,500 ~

2,500 mg/d (Cano et al., 2009). Therefore, patients may consume 200 g of low potassium lettuce (with a potassium content of 80 mg/100 g) without exceeding the recommended daily intake.

After cooked, potassium ion content of the vegetables will be reduced by 22 to 90% (Martinez-Pineda et al., 2016) or 30 to 50% (Burrowes et al., 2008) depends on cooking temperature and duration. Cook to reduce potassium content of the vegetables is good to Kidney patients, however, vegetables, after cooked will lose considerable amount of beneficial phytochemicals as well. For example, lettuces that have been cooked for 10 min will lose 40% of ascorbic acid and carotenoid and 30% of total phenol; the antioxidants also decrease significantly (Vinha et al., 2015).

Two experiments were conducted, the growing for each experiment were lasts for 6 weeks. The first focusing on using potassium-free nutrient solution for 3 weeks prior to harvest and the second focusing on using potassium-deficient nutrient solution for 2 weeks prior to harvest. Two standard operating procedures (SOP) for producing frill-ice lettuce with low potassium, sodium and nitrate contents were developed.

The 4K treatment from experiment 1 was chosen as the first SOP, which use nutrient solution with full strength potassium (100% K) for 4 weeks after sowing followed by 0% K for two weeks prior to harvest and leads to products with 56.2 g per plant in fresh weight and 36.96 mg of potassium per 100 g of lettuce. This SOP leads to the reduction of potassium content by approximately 90%, which is extremely low, however, the fresh weight also reduced by 48% compare with the lettuce grown under potassium-rich nutrient solution for 6 weeks. The lettuce produced following the first SOP can be served as the raw food by patients with kidney disease, preserving beneficial phytochemicals, and providing patients with a new low potassium dietary option.

The 0.75K treatment from experiment 2 was chosen as the second SOP, which use nutrient solution with full strength potassium (100% K) for 4 weeks after sowing followed by 50% K and 25% K during the 2nd week and the 1st week prior to harvest and leads to products with 100 g per plant in fresh weight and 172 mg of potassium per 100 g of lettuce. This SOP leads to the reduction of potassium content by 57.7 % and reduced fresh weight by only 4.7% compare with the lettuce grown under potassium-rich nutrient solution for 6 weeks. Assuming the potassium content can be reduced by half after cook, this SOP can be considered achieving the requirement of the low-potassium diet and most of all without scarify yield.

5. CONCLUSIONS

This study shows the potential of producing frill-ice lettuce with low potassium, sodium and nitrate contents in a PFAL using potassium-rich nutrient solution for the first

4 weeks and potassium-free (SOP 1) or potassium-deficient (SOP 2) nutrient solution two weeks prior to harvest. Both SOPs suggested can be used for the production of frill-ice lettuce served as the raw food and cooked vegetables for patients on a low potassium diet.

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