Development of Pre- and Postharvest Techniques to Produce Safe Baby Leaf Vegetables in a Plant Factory

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ABSTRACT

This study was conducted to develop preharvest techniques at each cultivation stage in a plant factory for improving the safety and yield of baby leaf vegetables and postharvest techniques for cleaning them. In the first chapter of this thesis, seeds of tat soi, romaine lettuce, and beet were sterilized with electrolyzed water (EW) and chlorine (CL) for 5 and 10 min. Microbial populations of the sterilized seeds decreased by sterilization treatments. *E.coli* and *Salmonella* spp. were not detected in any treatment. CL sterilization was most effective in reducing microbial populations. However, percentage of germination in all seeds was significantly decreased by CL treatments. When cultivation trays used in plant factory was irradiated with UVB for 20 min, the standard plate counts were less than 1 log CFU/g on cultivation tray. For improving the degree of safety and yield of baby leaf vegetables, the effects of different nutrient solutions on growth and quality of tat soi, romaine lettuce, and beet in a plant factory were examined. Fresh weights of tat soi and romaine lettuce increased the most when grown using nutrient solution of Yamazaki for lettuce (YMZK). Hunter's a values of tat soi and romaine lettuce decreased when using Korea Wonshi (KRWS) and Japan Enshi (JPES) nutrient solutions and the leaves were greener. Hunter's a values of beet increased when YMZK nutrient solution was used and the leaves were redder. Total phenolic contents of romaine lettuce were significantly increased in the treatment of KRWS. But, there was no significant difference in that of tat soi and beet. In the second chapter, the effects of surfactants and ultrasonic techniques for cleaning the harvested baby leaf vegetables were examined. Application of surfactants and ultrasonic treatment decreased the microbial population of tat soi to 1-2 log CFU/g. At the same time, rate of weight loss increased and visual index, and sogginess of tat soi decreased 9 days after storage. Hunter's L and b values of tat soi treated with surfactants and ultrasound increased during storage. No significant changes in Hunter's a value of tat soi was not found during storage. Results suggest that application of the pre- and postharvest techniques developed in the present study was effective for improving safety and yield of baby leaf vegetables.

Keyword: baby leaf production, food safety, plant factory, pre- and postharvest, surfactant, ultrasound

***Student Number*: 2014-20021**

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INTRODUCTION

As one-person households increased recently, consumption of ready-to-eat vegetables individually packaged in smaller portions has rapidly increased. Salad vegetable using various kinds of baby leaf vegetables are available in market. Fresh-cut salads take up 55.2% market share of domestic salads business, while baby leaf vegetables and sprouts hold 37.8, and 7.0%, respectively (Lee, 2013). Total retail sales of baby leaf vegetables were estimated 24 billion won in 2013 (Choi, 2013). Based on this trend, the future market of salad using baby leaf vegetables in the food service industry, as well as processed food for home use, is expected to expand.

Baby leaf vegetables are harvested in young stage before reaching maturation. The advantages of baby leaf vegetables are that they have the shorter cultivation period (20-30 days) and contain the higher contents of vitamins, minerals, and antioxidants (Ogimoto, 2000). The fiber of baby leaf vegetables has the advantage of being soft and easy to eat without tough texture.

However, the soft texture of baby leaf vegetables often brings the difficulties in postharvest handling. Soft texture is likely to be broken or easily soften by the washing and dewatering process. Even just a little softening in the progress of processing and storage can cause loses in commercial value. Texture decaying or softening is a factor in limiting the market ability of baby leaf vegetables (Choi, 2013). More sterile cultivation methods can reduce requirement for intensive washing and significantly improve the quality and marketing ability of baby leaf vegetables.

Most baby leaf vegetables are grown hydroponically in greenhouses. Consumers prefer the vegetables free from the dangers of pesticides, pollutants, and microbes (Kurihara et al., 2014). The cultivation of baby leaf vegetables in a plant factory will be able to meet the needs from consumers about food safety.

Plant factory is a closed cultivation system where the plants are grown in separated from the external environment. Vegetables can be massively produced year-round and plant growth and functional substances of vegetables can also be improved by controlling nutrient solution or environmental conditions such as air temperature, light intensity, light quality, photoperiod, and so on (Chen et al., 2014; Kang et al., 2013; Wu et al., 2012). It is easy to create an environment with low microbial contamination in the plant factory. Little researches, however, has been reported on the microbial contamination control in plant factory. The objectives of this study were to develop preharvest techniques at each cultivation stage in a plant factory for improving the safety and yield of baby leaf vegetables

(Chapter 1), and to develop postharvest techniques for cleaning baby leaf vegetables (Chapter 2).

LITERATURE REVIEW

Microbial decontamination on seeds by treatment of slightly acidic electrolyzed water

Illness related to contamination of microorganisms such as *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Listeria monocytogenes*, and *Enterococcus faecalis* on seed has been increasing in Canada, USA, the UK, and Japan, which raised the interest in method of reducing microorganism on seeds (Olaimat and Holley, 2012; Soon et al., 2013). These methods include chemical and physical treatments. Chemical treatments use chemicals such as hydrogen peroxide, hypochlorite, chlorine dioxide, calcium hydrogen peroxide, ethanol, etc. to kill the microorganism. Physical treatments employ physical factors such as heat, irradiation, ultrasound, etc. Combination of different chemical and physical methods has also been performed with the use of high pressure, temperature, and chemical treatment (Peñas et al., 2010), ultrasound, heat, and chemical treatment (Scouten and Beuchat, 2002). Among the chemical methods, slightly acidic electrolyzed water (SAEW) is found to have good potential in microbial decontamination on seeds (Zhang et al., 2011). When the SAEW is generated in a neutral pH 5.0-6.0 by electrolysis of dilute hydrochloric acid in the absence of the membrane, the produced hypochlorous acid has strong antimicrobial activity (Yoshifumi, 2003). The advantages of SAEW are very low toxic side effect to human and less corrosive to materials than other chemicals (Guentzel et al., 2008).

Decontamination by ultrasound washing techniques

The food-borne bacteria including *Bacillus, Salmonella, Listeria, Staphylococcus*, and *Escherichia* attached to vegetables caused several outbreaks (Sinde and Carballo, 2000; Ryu and Beuchat, 2005). Thus, disinfection cleaning technologies for reducing microbial contamination and extending shelf-life without deterioration in product quality are continuously being investigated.

Washing is a main method that helps to remove the soil as well as microbes and contaminants in fresh products. It is important for controlling quality and freshness after harvesting, but excessive washing causes damage to the vegetables and cross-contamination between washed and contaminated products (Luo et al., 2011; Olaimat and Holley, 2012). Although chlorine is still widely used to wash vegetables, the use of chlorine may be harmful due to generated carcinogenic by-products such as trihalomethanes, chloroform, and haloacetic acids when chlorine reacts with organic matter (Al-Zenki et al., 2012; Hua and Reckhow, 2007; Ölmez and Kretzschmar, 2009).

Ultrasound has antimicrobial effects with intracellular cavitation that cause physical loses in the selectivity of the membranes and makes a them thinner while increasing the permeability of the cell membrane, chemically destroys the DNA with the free radical produced by cavitation (Butz and Tauscher, 2002; Fellows, 2000; Sams and Feria, 1991). The application of ultrasound is an alternative decontamination technology to chlorine as a safe, non-toxic, and environmentally friendly washing method which helps to improve microbial safety and extending shelf-life in food without changes of nutritional, sensory qualities (Alegria et al., 2009; Cao et al., 2010 ; Kentish and Ashokkumar, 2011). There have been a number of reports about applications of ultrasound in the cleaning and disinfecting of some fruits and vegetables (Alexandre et al., 2012; Elizaquivel et al., 2012; Huang et al., 2006; Sagong et al., 2011; Seymour et al., 2002; Zhou et al., 2009). However, few researches were reported on the ultrasonic wave decontamination techniques for baby vegetables or sprouts.

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CHAPTER 1

Development of Preharvest Techniques for Improving Safety and Yield of Baby Leaf Vegetables in a Plant Factory

INTRODUCTION

Vegetables can become contaminated with pathogens that can cause human diseases while during the process of cultivation, harvesting, transporting, or distribution. Specifically, potential preharvest factors of contamination are seed, soil, irrigation water, dust, insects, and human handling during cultivation (Beuchat, 1996; Burnett and Beuchat, 2001).

The seeds seem to be the main cause of contamination related to foodborne illness in the sprouts. Furthermore, the germination process conditions are ideal environment for the pathogens to grow exponentially (NACMCF, 1999). High levels of chlorine can be effective in killing pathogens on seeds but are also detrimental to the quality of seeds (Kim et al., 2003). To reduce or eliminate the pathogens on seeds, electrolyzed water (EW) is regarded as one of the promising substitutes for chlorine (Ding et al., 2010; Zhang et al., 2011).

Fertilization allows the improvement of yield and nutritional quality of plants by a practical and effective preharvest way (Fallovo et al., 2009). However, there may be no nutrient solution specified for baby leaf vegetables, while numerous nutrient formulas are available for leafy and fruit vegetables. Therefore, it is crucial to develop a decontamination method to effectively reduce microorganism population on seeds and an optimal nutrient solution composition for baby leaf vegetable in a plant factory. The objective of the study in this chapter was to develop preharvest techniques at each cultivation stage in a plant factory for improving the safety and yield of baby leaf vegetables.

MATERIALS AND METHODS

Seed sterilization treatments

Unsterilized seeds of tat soi, romaine lettuce, and beet were used. In tap-water (TW) treatment seeds were rinsed with tap water for 5 min. Electrolyzed water (EW) was generated using an electrolyzed water generator (HCH240, Siontech, Korea). Available chlorine concentration of EW was 10, 20, and 30 ppm and that of chlorine (CL) was 2000 ppm. Seeds were soaked in solutions for 5 and 10 min, except in the not-washed (NW) treatment set as a control.

Analysis of Microbial populations

One gram of sample in 9 mL of distilled water was homogenized. And then, sample was ten-fold diluted by distilled water. 1 mL of the sample was placed on the 3M Petrifilm (3M, St. Paul, MN, USA). Petrifilms were incubated at 35-7^{o}C for 24 ± 2 hours to determine standard plate count and *E. coli*. For *Salmonella*, 1 mL of the sample was placed on Salmonella Shigella Agar (Difco Laboratories Inc., Detroit, MI, USA) with incubation at 35-7^{o}C for 24 ± 2 hours.

Determination of seed germination

One hundred seeds were placed on 90 mm diameter petri dish with 2 pieces of saturated filter paper No.2 (Whatman International Ltd., Maidstone, Kent, UK). The petri dishes were sealed with parafilm to keep the filter papers moist and placed in the dark at 25^{o}C for 3 days. The number of germinated seeds was counted, which were chosen with the radicles becoming visibly protruded from the seed coat by at least 2 mm, and the percentage of germination was calculated.

UVB irradiation treatment

The UVB irradiation was carried out by treating with of 4.2 W·m^{-2} for 10, 20, and 30 min. Unwashed cultivation tray was screened in UV chamber. After UVB irradiation treatment, microbial populations were immediately measured.

Nutrient solution treatment

The seeds of tat soi, romaine lettuce, and beet were sown in urethane sponges and cultivated for 14 days in a plant factory. Light intensity and photoperiod were 110 μmol·m-2·s-1 and 16 h, respectively; and air temperature was maintained at 25/20oC during photo/dark-period. Tap water was used for irrigation for 7 days after sowing, and then plants were irrigated tap water (TW) and the nutrient solutions of Korea Wonshi (KRWS), Japan Enshi (JPES), and Yamazaki's lettuce (YMZK) for 7 days. Composition of nutrient solutions for KRWS was NO_3-N 14.0, NH_4-N 1.0, PO_4-P 3.0, K 6.0, Ca 8.0, Mg 4.0, SO_4-S 4.0 me·L^{-1}, EC 2.3 mS·cm^{-1}, pH 6.5 (Park and Kim, 1998); for JPES was NO_3-N 16.0, NH_4-N 1.3, PO_4-P 4.0, K 8.0, Ca 8.0, Mg 4.0, SO_4-S 4.0 me·L^{-1}, EC 2.2 mS·cm^{-1}, pH 6.5 (Hori, 1966); and for YMZK: NO_3-N 6.0, NH_4-N 0.5, PO_4-P 1.5, K 4.0, Ca 2.0, Mg 1.0, SO_4-S 1.0 me·L^{-1}, EC 0.85 mS·cm^{-1}, pH 6.0-6.5 (Yamazaki, 1982).

Determination of Hunters' values

The color of leaf samples was measured with a colorimeter (CR-400, Minolta, Osaka, Japan) to determine color changes, which was indicated by three parameters: L (ranging from 0 to 100, 'black to white'), a (ranging from 0 to 100, 'red to green'), and b (ranging from 0 to 100, 'yellow to blue') values.

Determination of total phenolic content

Total phenolic content (TPC) was estimated as gallic acid equivalent (GAE) using the Folin–Ciocalteu (FC) spectrophotometric method, established by Singleton and Rossi (1965). 1 g of sample in 10 mL of extraction solvent (acetone: methanol: distilled water: acetic acid = 40: 40: 20: 10) was homogenized and placed under 60°C for 1 h. The mixture was filtered with 0.45μm syringe filter (Whatman International. Ltd., Maidstone, Kent, UK). 1 mL of 10% FC reagent and 7.5% of Na_2CO_3 were added. The reaction mixture was allowed to react at room temperature for 2 h. Finally, absorbance was measured at 725 nm with a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). Results were expressed in milligrams of gallic acid equivalent to 100 g fresh weight of sample.

Statistical analysis

Data were analyzed using SAS 9.4 version (SAS Inst. Inc., Cary, NC, USA) for Duncan's multiple range test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

Changes in microbial populations and seed germination

Microbial populations of sterilized seeds were decreased by sterilization treatments. *E.coli* and *Salmonella* spp. were not detected on seeds of baby leaf vegetables in any treatments. Standard plate counts of tat soi seeds after treating with tap water showed nearly 1 log CFU/g lower than control. EW treatments also reduced more than 2 log CFU/g of microbial populations of tat soi seeds (Table 1-1). Standard plate counts of romaine lettuce seeds after treating with tap water showed nearly 1 log CFU/g reduction. EW treatments had little effect on reducing microbial changes (Table 1-2). Yokoyama et al. (2007) reported that structural differences in cell walls between Gram-positive and Gram-negative bacteria are to account for the observed differences due to structural changes and destruction of the cell walls that occurs upon treatment with electrolyzed water.

Similarly, compared to tat soi results, standard plate counts of beet seeds after treating with tap water also showed nearly 1 log CFU/g lower than control. EW treatments also gave more than 2 log CFU/g reduction on microbial populations of beet seeds (Table 1-3). There was no difference compared to control in seed germination of tat soi and romaine lettuce, except for CL treatments (Figs. 1-1 and 1-2). CL sterilization treatments were most effective to reduce microbial populations. In CL-10 treatment, microbial populations in seeds of tat soi, romaine lettuce, and beet were 2.43, 2.56, and 2.99 log CFU/g reduced, respectively (Tables 1-1, 1-2, and 1-3). However, the percentage of germination in all seeds was significantly decreased in CL-10 treatments (Figs. 1-1, 1-2, and 1-3), which was similar to results reported by Derso and Feyissa (2015), Yildiz (2002), and Yadav and Singh (2011).

Issa-Zacharia et al. (2010) demonstrated that EW treatment significantly reduced the populations of total aerobic mesophilic bacteria, *E.coli*, and *Salmonella* spp. from Chinese celery, lettuce and radish sprouts. Microorganism development could be reduced because EW has antimicrobial effect against most viable bacteria cells, pathogenic and non-pathogenic bacteria and spore, virus and fungus (Kim et al., 2000a; Hotta and Suzuki, 1999; Venkitanarayanan et al., 1999 a, b). The active factors responsible for the bactericidal effect have been reported to be chlorine related substances, namely chlorine (Cl_2), hypochlorous acid (HOCl), and hypochlorous acidic ion (ClO^-) (Izumi, 1999; Kim et al., 2000 a, b; Kim et al., 2003; Park et al., 2001). Increasing the treatment time adversely affect the physical appearance and nutritional content of products (Park et al., 2008).

Previous studies demonstrated that EW at low chlorine concentration (10 to 30 $mg \cdot L^{-1}$) possess similar or higher bactericidal activity than sodium hypochlorite solution (100 to 200 $mg \cdot L^{-1}$) (Cao et al., 2008; Deza et al., 2005; Kim et al., 2000a, b; Issa-Zacharia et al., 2009). Results indicate that EW10-10 treatment can be appropriate to be applied for preharvest cultivation technique in a plant factory.

Table 1-1. Effect of seed sterilization treatments for tat soi on microbial population and colony development of *E. coli* and *Salmonella* spp.

Treatment	Standard plate count (log CFU/g)		*E. Coli*	*Salmonella* spp.
NW	5.41	a[z]	N.D.[y]	N.D.
TW-5	4.56	b	N.D.	N.D.
TW-10	4.35	c	N.D.	N.D.
EW10-5	3.39	d	N.D.	N.D.
EW20-5	3.31	de	N.D.	N.D.
EW30-5	3.40	d	N.D.	N.D.
EW10-10	3.30	de	N.D.	N.D.
EW20-10	3.29	de	N.D.	N.D.
EW30-10	3.38	d	N.D.	N.D.
CL-5	3.25	e	N.D.	N.D.
CL-10	2.98	F	N.D.	N.D.

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

[y]Not detected.

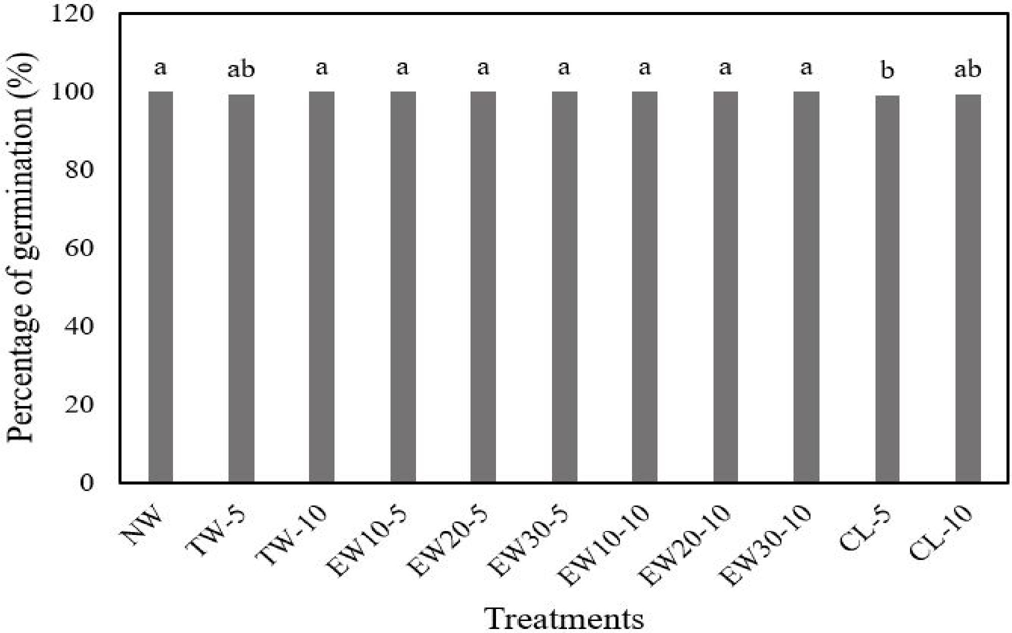

Fig. 1-1. Germination rates of tat soi seeds treated with tap water, electrolyzed water, and chlorine for 5 and 10 min.

Table 1-2. Effect of seed sterilization treatments for romaine lettuce on microbial population and colony development of *E. coli* and *Salmonella* spp.

Treatment	Standard plate count (log CFU/g)		*E. Coli*	*Salmonella* spp.
NW	5.70	a[z]	N.D.[y]	N.D.
TW-5	4.78	e	N.D.	N.D.
TW-10	4.41	f	N.D.	N.D.
EW10-5	5.04	d	N.D.	N.D.
EW20-5	5.51	b	N.D.	N.D.
EW30-5	5.18	c	N.D.	N.D.
EW10-10	5.03	d	N.D.	N.D.
EW20-10	5.50	b	N.D.	N.D.
EW30-10	5.19	c	N.D.	N.D.
CL-5	3.46	g	N.D.	N.D.
CL-10	3.14	h	N.D.	N.D.

[Z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

[y]Not detected.

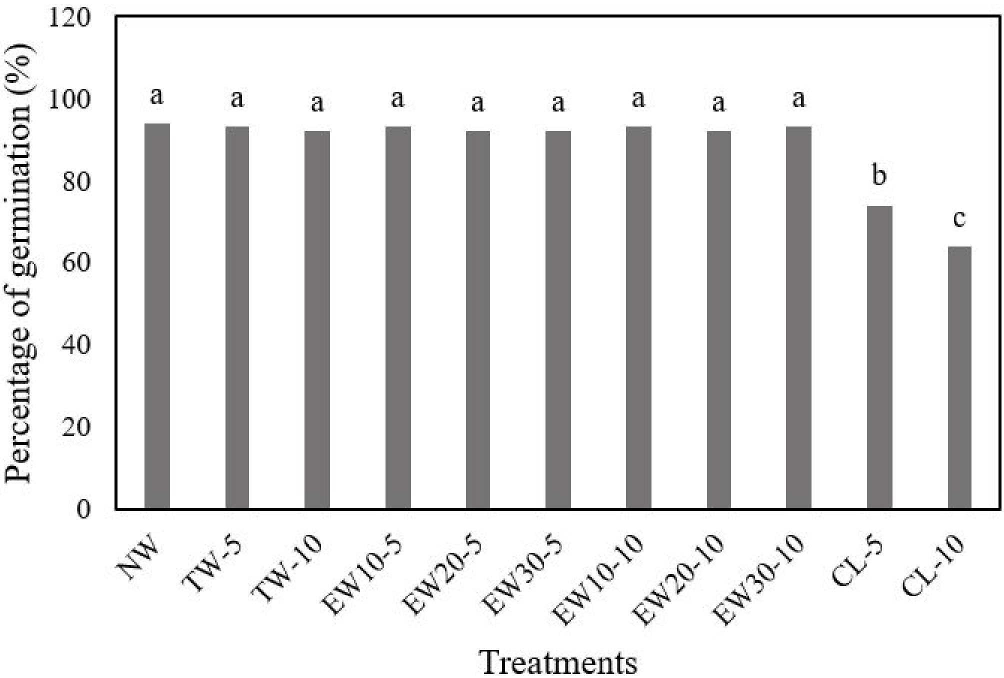

Fig. 1-2. Germination rates of romaine lettuce seeds treated with tap water, electrolyzed water, and chlorine for 5 and 10 min.

Table 1-3**.** Effect of seed sterilization treatments for beet on microbial population and colony development of *E. coli* and *Salmonella* spp.

Treatment	Standard plate count (log CFU/g)		*E. Coli*	*Salmonella* spp.
NW	5.63	a[z]	N.D.[y]	N.D.
TW-5	4.38	b	N.D.	N.D.
TW-10	4.17	c	N.D.	N.D.
EW10-5	3.31	f	N.D.	N.D.
EW20-5	3.49	de	N.D.	N.D.
EW30-5	3.35	ef	N.D.	N.D.
EW10-10	3.26	f	N.D.	N.D.
EW20-10	3.53	d	N.D.	N.D.
EW30-10	3.34	ef	N.D.	N.D.
CL-5	2.94	g	N.D.	N.D.
CL-10	2.64	h	N.D.	N.D.

[Z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

[y]Not detected.

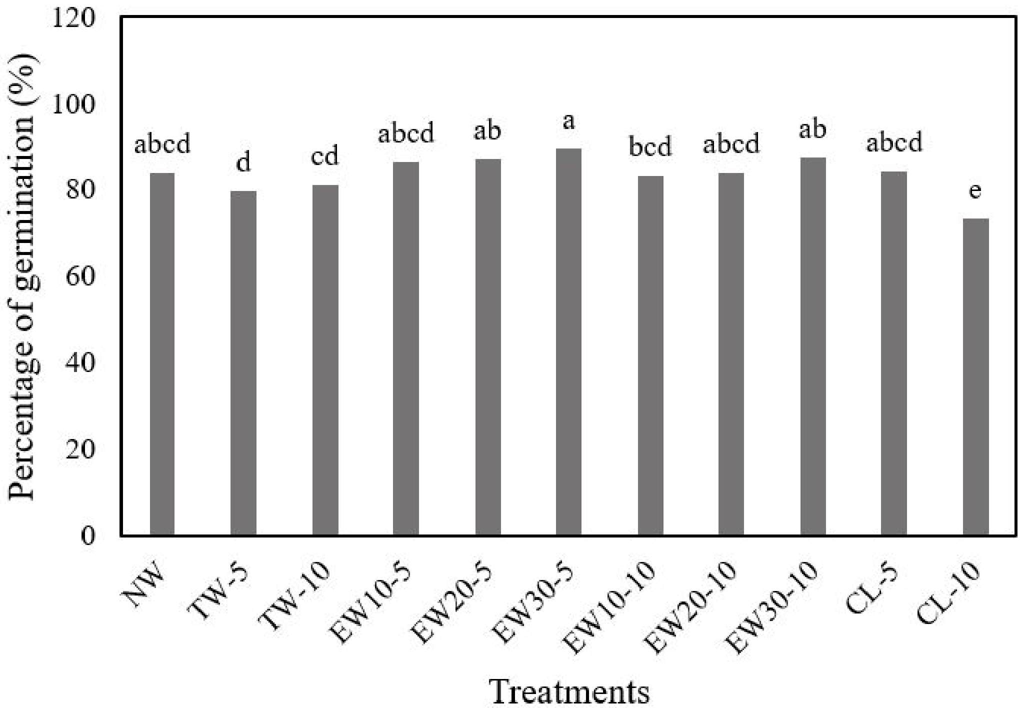

Fig. 1-3. Germination rates of beet seeds treated with tap water, electrolyzed water, and chlorine for 5 and 10 min.

Changes in microbial population of cultivation tray by UVB irradiation

Effect of UVB irradiation on reducing microbial populations of cultivation tray was investigated. Initial microbial population of cultivation tray was 1.95 CFU/g. After UVB irradiation for 10 min on cultivation tray, microbial populations of cultivation tray had dropped by more than half of the initial population. When cultivation tray was irradiated with UVB for 20 min, standard plate counts gave the result of under log 1 CFU/g per tray (Fig. 1-4).

The UV light, with some precautions, is easy to use and lethal to most types of microorganisms (Bintsis et al., 2000). The wavelengths between 220 and 300 nm are considered germicidal against microorganisms such as bacteria, viruses, protozoa, molds and yeasts, and algae (Morgan, 1989; Sizer and Balasubramaniam 1999; Bintsis et al., 2000). UVB initiates a photo-oxidative reaction that changes anti-ROS-sensitive signaling pathways, which increases the cellular level of ROS (Punnonen et al., 1991). Toxic products mainly derived from lipid peroxidation and protein carbonylation under UVB exposure accelerate further oxidative damage. Liltved and Landfald (2000) reported that the radiation absorbed by DNA may stop cell growth and lead to cell death.

Results suggest that prior to cultivating baby leaf vegetables the cultivation trays should be UVB irradiation for 20 min to effectively reduce the microbial contamination.

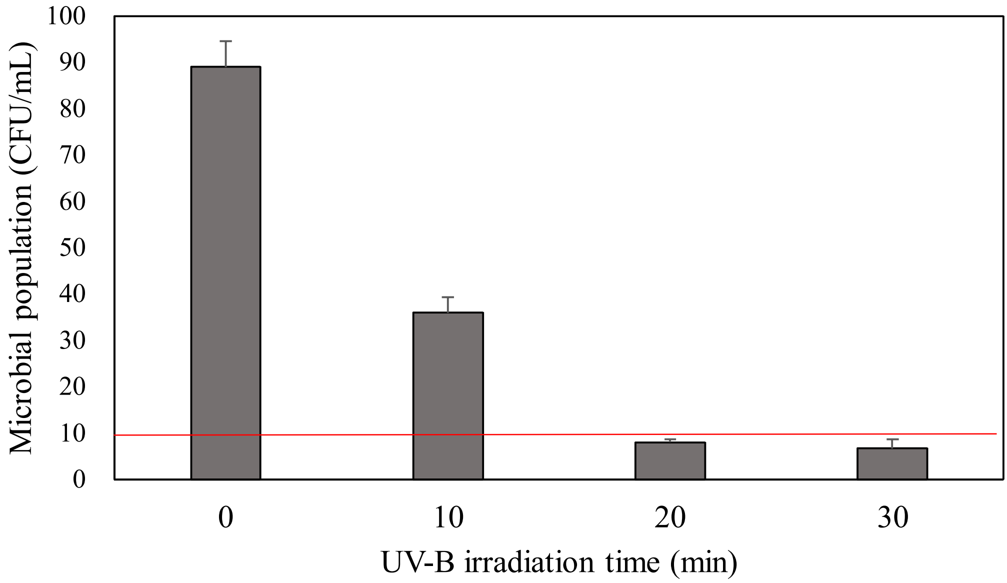

Fig. 1-4. Decrease in microbial population of cultivation tray by UVB irradiation treatment.

Changes in quality and yield of baby leaf vegetables as affected by composition of nutrient solutions

Fresh weights of tat soi and romaine lettuce were significantly increased in YMZK treatment and reached 283 and 267 mg per plant, respectively. No significant difference in fresh weight of beet was observed among in all the nutrient solution treatments (Table 1-4).

Seo et al. (2009) reported that EC of nutrient solution affects not only lettuce growth, but also concentration in lettuce. Fresh weight production of the three lettuce varieties 'Tom Thumb', 'Paris Island' and 'Ice Queen' decreased with increasing EC level of the nutrient solutions (Abou-Hadid et al., 1996). Those results indicated that the EC of the nutrient solution significantly affected lettuce growth including visual quality and mineral concentration in the plants (Abou-Hadid et al., 1996; Serio et al., 2001).

Hunter's a values of tat soi and romaine lettuce decreased in KRWS and JPES treatments indicating that tat soi and romaine lettuce had more green leaves. Hunter's a value of beet increased in YMZK treatment showing that beet had more red leaves (Table 1-5). Chlorophyll content of plants grown in the EC 2.0

treatment was significantly higher than the other treatments. The result of tat soi had the same tendency. Carlo et al. (2009) also showed that leaf color parameters L∗ (lightness), a∗ (greenness) and b∗ (yellowness) were highly influenced by nutrient solution concentration. The chlorophyll content and a∗ value of leafy lettuce increased with increasing nutrient solution concentration.

However, an increase in total salt concentration in the nutrient solution leads to osmotic stress and consequently to a decrease in N uptake, which may also affect the biosynthesis of plant metabolites, including chlorophyll (Carlo et al., 2009). A similar response of chlorophyll degradation in relation to biotic and abiotic stresses such as water stress, heat stress, insect feeding and aging has been reported previously (Hörtensteiner, 2006; Majunmdar et al., 1991; Ni et al., 2001).

There are many reports showing that nitrogen deficiency induces increased levels of anthocyanins and other flavonoids in Arabidopsis (Hsieh et al., 1998; Martin et al., 2002; Scheible et al., 2004). Nitrogen deficiency enhances expression of specific MYB and bHLH transcription factors and accumulation of end products in the flavonoid pathway (Lea et al., 2007). Total phenolic contents of romaine lettuce were significantly increased in KRWS treatments. In KRWS, JPES, and YMZK treatments, total phenolic contents of romaine lettuce were

12.9, 10.7, and 7.9 mg GAE g FW, respectively. But, there were no such differences as affected by composition of nutrient solutions in tat soi and beet (Table 1-6). Results might be caused by osmotic stresses from high EC of nutrient solutions which agrees with results of Ehret et al. (2013) reporting that concentrations of antioxidants increased when EC of nutrient solution increases.

Table 1-4. Effect of composition of nutrient solutions on fresh weight of tat soi, romaine lettuce, and beet cultivated in a plant factory.

Treatment	Fresh weight (mg/plant)		
	Tat soi	Romaine lettuce	Beet
TW	47 c[z]	41 c	40 b
KRWS	210 b	230 a	154 a
JPES	206 b	152 b	143 a
YMZK	283 a	267 a	143 a

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

Table 1-5. Effect of composition of nutrient solutions on Hunter's L and a values of tat soi, romaine lettuce, and beet cultivated in a plant factory.

Treatment	Tat soi		Romaine lettuce		Beet	
	L	a	L	a	L	a
TW	40.7 a[z]	-9.4 ab	48.1 a	-5.6 a	38.4 b	-2.3 a
KRWS	34.2 c	-10.9 bc	39.3 b	-10.8 b	42.3 a	-8.2 bc
JPES	38.5 ab	-11.7 c	41.4 b	-9.7 b	42.7 a	-9.2 c
YMZK	37.6 b	-8.2 a	39.6 b	-9.2 b	43.6 a	-7.4 b

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

Table 1-6. Effect of composition of nutrient solutions on total phenolic contents of tat soi, romaine lettuce, and beet cultivated in a plant factory.

Treatment	Total phenolic content (mg GAE g FW)		
	Tat soi	Romaine lettuce	Beet
TW	12.5 a[z]	10.3 b	9.6 a
KRWS	12.9 a	12.9 a	9.7 a
JPES	12.3 a	10.7 b	10.2 a
YMZK	11.5 a	7.9 c	9.0 a

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

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CHAPTER 2

Application of Surfactant and Ultrasonic Techniques for Cleaning Baby Leaf Vegetables

INTRODUCTION

As consumption of ready-to-eat salad vegetable has increased, salad vegetables became widely used in many cuisines. Recently, as food poisoning associating with fresh vegetables has been occurring more frequently around the world than before, consumers are more concerned with safety.

The high level of microbial contamination on salad vegetables was reported to be at an average of log 1.5-8.4 CFU/mL (Jo et al., 2011) in Korea. However, agricultural products has not been subject to microbial management, yet. Only foodborne pathogens such as *E. coli*, *Salmonella* spp., *Vibrio parahaemolyticus*, and *Bacillus cereus* in ready-to-eat vegetables are managed (KFDA, 2009).

Washing is an important technique in fresh product processing to reduce microbial population. Various studies have shown that many sanitizing agents, such as chlorine, organic acids, ozone and some surfactants are excellent antimicrobials. The most commonly used agent to clean the vegetables in chlorine because of its good efficiency. When the chlorine is consumed with the presence of organic matter turbidity of the wash water increases (Luo et al., 2012; O'Beirne and Zagory, 2009). The presence of organic matters in wash water can also enhance formation of chloroform ($CHCl_3$), haloacetic acids or other trihalomethanes (THM), all of which are known to be harmful to human health (Artés et al., 2009).

The alternative disinfectants to chlorine such as organic acids, ozone are less effective than chlorine to reduce microbial contamination of vegetables. The combination of a chemical and physical wash process, such as sonication, has been tested for improving the efficacy of a sanitizer (Zhou and Luo, 2009; Zhou et al., 2012). However, when used beyond certain critical concentrations, washing treatments produced negative impact on the quality of fresh product. For example, quality deteriorations such as browning, tissue softening, color changes, and overall poor appearance are usually observed (Garcia et al., 2003). The objective of the study in this chapter was to investigate the effects of combining surfactants with sonication on improving microbial safety and quality of baby leaf vegetables.

MATERIALS AND METHODS

Plant materials and storage conditions

Freshly harvested tat soi was purchased from an agriculture corporation company (Pyeongtaek, Korea) and stored at 4°C before experiment. The experiment was conducted within 2 days of purchase. After washing treatments, the surface water remaining on the leaves of the plants was immediately removed by centrifuging with a manual salad spinner. One gram of dewatered plants was packaged in polypropylene plastic film bags (18 cm × 20 cm) and stored in chamber at 5°C for 9 days. The air temperature and relative humidity were monitored using a data logger (ALMEMO 2590-9, Ahlborn, Holzkirchen, Germany).

Surfactant treatment

Surfactant Span 20 (Sigma, St. Louis, MO, USA) was used in this experiment. Various concentrations of the surfactant such as 50 and 100 ppm were dissolved in sterile distilled water. 15 g of sample was immersed in surfactant solutions in a 500 mL glass beaker for 10 min.

Ultrasonic treatment

After surfactant treatment, sample was immersed with 500 mL of distilled water in the beaker. Ultrasonic treatment was performed with an ultrasonic cleaner (JAC-4020, Kodo Technical Research Co., Ltd, Hwaseong, Korea). The water in the bath was filled with 10 L of tap water. Ultrasound treated at frequency of 40 kHz with power of 400W at 15°C for 10 min. The process was performed one, two or three times, each time the water in the beaker was renewed.

Analysis of standard plate counts

One gram of sample in 9 mL of physiological saline water was homogenized in a stomacher (Easy Mix, AES Chemunex, Rennes, France). And then, sample was ten-fold serially diluted by physiological saline. 1 mL of the diluted sample was placed on the 3M Petrifilm (Petrifilm, 3M, St. Paul, MN, USA). The petrifilms were incubated at 35-37°C for 24 ± 2 hours to determine standard plate counts.

Determination of rate of weight loss

Weight loss was determined by comparing sample weights with their previous recorded weights at three days intervals and rate of weight loss is presented as percentage of weight loss during storage compared to initial weight before storage.

Determination of Hunters' values

The color of sample was measured with a colorimeter (CR-400, Minolta, Osaka, Japan) to determine color change, which was indicated by three parameters: L (ranging from 0 to 100, 'black to white'), a (ranging from 0 to 100, 'red to green'), and b (ranging from 0 to 100, 'yellow to blue') values.

Determination of quality evaluation

Visual index was assessed using parameters modified from Guan et al. (2010). Overall visual quality was rated on a 10 to 1 scale: 10 = excellent, essentially free from defects; 7 = good, minor defects, not objectionable; 5 = fair, slightly to moderately objectionable defects, lower limit of sales appeal; 3 = poor, excessive defects, limit of salability; 1 = extremely poor, not usable. Sogginess was presented as percentage of fully soggy leaves in a sample.

RESULTS AND DISCUSSION

Changes in microbial populations of tat soi by surfactants and ultrasonic treatments

Microbial population of tat soi was reduced by immersing them into surfactant solution. Microbial reduction was 1.13-1.4 log CFU/g in 50 ppm of Span 20 treatments and 1.06-1.59 log CFU/g in 100 ppm of Span 20 treatments. When tat soi was immersed in surfactant solution without ultrasound washing, there was no significant difference in regard to the number of washing times (Fig. 2-1).

The reduced number of microorganisms in surfactant treatment is associated with the bacterial detachment capability of the surfactants. Paul and Jeffrey (1985a, b) demonstrating that surfactants detached the bacteria by reducing the hydrophilic interaction. These were consistent with NaCl/$NaHCO_3$ solution removing *E.coli* O157:H7 from cut lettuce leaves (Janes et al., 1999) and surfactant/bicarbonate solution detaching *E.coli* O157:H7 from lettuce leaves

(Hassan and Frank, 2003). Amato (1993) also proved that the combination of surfactant and sanitizer has more efficacy than using sanitizer alone.

Microbial reduction increased with the number of washing by surfactants and ultrasound treatments. As the number of washing time was increased from 1 to 3 times, microbial population was reduced 1.14, 1.72, and 2.04 log CFU/g in 50 ppm of Span 20 treatments with ultrasound, respectively, and 0.95, 1.31, and 1.73 log CFU/g in 100 ppm of Span 20 treatments with ultrasound, respectively (Fig. 2-1).

Consistent with these results, Sagong et al. (2013) also showed that surfactant and ultrasound treatments could reduce *B. cereus* spores. Microorganisms were cleaned by detaching and dispersing with the cavitation effect of the ultrasonic waves (Dehghani, 2005; Frizzell, 1988; Mahvi et al., 2005; Russell, 2001).

Surfactant does not need high concentration to be effective. When using in an appropriate concentration of surfactant, it can achieve good bacterial reduction by detachment ability. Washing with an ultrasound also can enhance the level of microbial reduction. Further research should consider whether more microorganisms can be reduced by increasing the number of washing times with ultrasound.

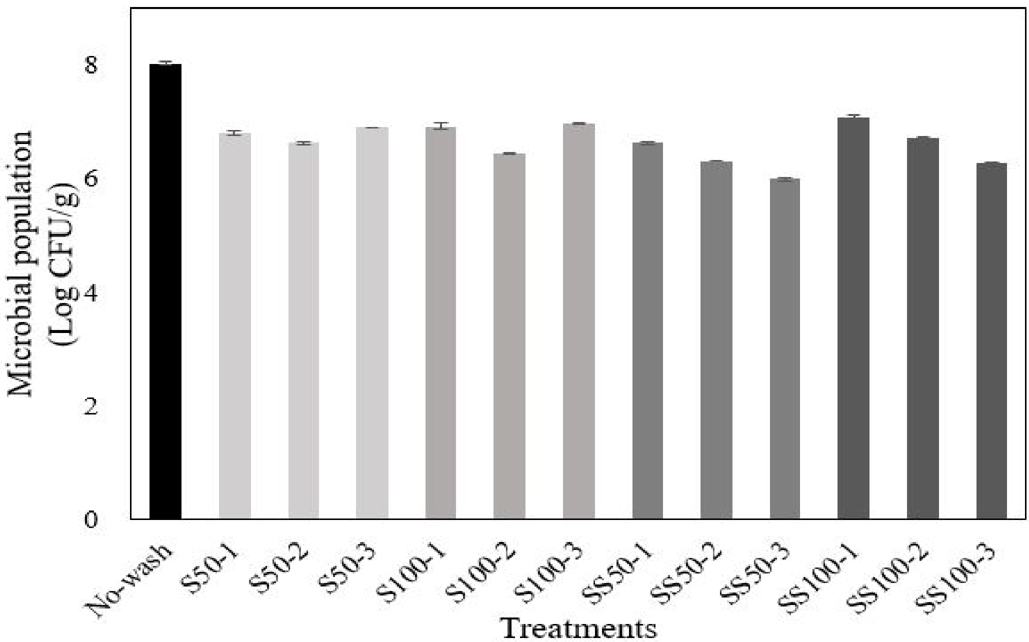

Fig. 2-1. Changes in microbial populations of tat soi by treatment of surfactants and ultrasonic wave.

Changes in plant quality of tat soi treated with surfactants and ultrasonic waves during storage

The weight loss of tat soi treated with surfactants and ultrasound increased more than 10% in all treatments except for control 9 days after storage. The lowest percentage of weight loss was observed in control. Especially, after 3 times of washing with ultrasound treatment, the weight loss of tat soi was 2-fold increased as 20.7 and 19.0% in 50 ppm and 100 ppm of Span 20, respectively (Table 2-1).

Increasing weight loss of tat soi during storage was significantly affected by ultrasonication. Cytoplasmic membrane from the cell wall by the irradiation of ultrasound leaked while the cell wall becomes thinner (Alliger, 1975). Physical damages could bring about to be vulnerable to decay by microorganisms and be decreased water content and vitamin C consistently during storage (Sharma and Singh, 2000; Kader, 2002).

Hunter's L and b values of tat soi treated with surfactants and ultrasonication increased during storage. No distinct tendency showed up within changes in Hunter's a value of tat soi during storage (Table 2-2).

No significant difference was observed 3 days after storage among any treatments. Visual index of tat soi was more decreased 3 times of washing 9 days after storage in 100 ppm of Span 20 surfactant with ultrasound (Table 2-3). The lower sensory quality during storage could be explained by reduced chlorophyll. Leafy vegetables loss greenness as senescence with the degradation of chlorophyll (Chl) (Yamauchi and Watada, 1991). Chlorophyllase activity, which catalyzes the release of the phytol chain from Chl to form chlorophyllide (Chd), has been reported to increase with yellowing in barley and oat leaves (Rodriguez et al., 1987; Sabater and Rodriguez, 1978).

No significant difference was observed until 3 days after storage in all treatments. Sogginess of tat soi was remarkably increased as 43% 9 days after storage in 100 ppm of Span 20 surfactant 3 times of washing with ultrasound treatment (Table 2-4). The results are consistent with those of Salgado et al. (2014) reporting the negative effects on the sensory quality of lettuces during storage in a combined wash treatment of sanitizer, surfactant, and ultrasonication. Washing vegetables using a surfactant and applying physical shearing forces with ultrasound waves to reduce microbial contamination can be useful to maintain microbial safety and quality of vegetables.

Table 2-1. Changes in weight loss of tat soi treated with surfactants and ultrasonic wave 9 days after storage.

Treatment	No. wash	Rate of weight loss (%)
No-wash	0	7.87 c[z]
Span 50 ppm	1	10.60 bc
	2	10.33 bc
	3	15.03 b
Span 100 ppm	1	14.07 b
	2	12.47 bc
	3	14.37 b
Span 50 ppm with ultrasound	1	12.07 bc
	2	11.03 bc
	3	20.77 a
Span 100 ppm with ultrasound	1	11.63 bc
	2	13.17 b
	3	19.27 a

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

Table 2-2. Changes in Hunter's L, a, and b values of tat soi treated with surfactants and ultrasonic wave during storage.

Treatment	No. Wash	Hunter's L, a, and b values on storage time								
		Day 0			Day 3			Day 9		
		L	a	b	L	a	b	L	a	b
No-wash	0	34.98 c[z]	-11.06 abc	12.22 cd	41.71 def	-10.98 b	13.13 ef	42.59 bcd	-7.49 a	15.15 bc
Span 50 ppm	1	39.11 ab	-12.28 bcd	14.60 abcd	43.85 cd	-12.39 cd	14.71 cde	42.86 bcd	-12.00 bcde	16.72 bc
	2	37.92 abc	-11.84 abcd	13.16 bcd	43.55 cde	-11.03 b	14.03 de	48.48 ab	-12.16 cde	16.62 bc
	3	38.19 abc	-11.00 abc	12.42 cd	45.49 bc	-11.47 bc	16.14 bc	45.34 bcd	-11.54 bced	16.90 bc
Span 100 ppm	1	37.12 bc	-13.33 cd	15.81 abc	42.79 def	-11.43 bc	12.95 ef	47.90 ab	-11.33 bcd	18.84 ab
	2	34.89 c	-10.41 ab	12.32 cd	39.44 gh	-9.62 a	11.01 g	53.44 a	-11.78 bcde	23.47 a
	3	41.03 ab	-13.54 cd	17.07 ab	45.03 c	-14.13 e	17.06 ab	46.21 bc	-9.40 ab	14.99 bc
Span 50 ppm with ultrasound	1	41.08 ab	-10.93 abc	13.20 bcd	47.42 ab	-14.51 e	18.72 a	46.59 bc	-14.22 e	18.82 ab
	2	37.18 bc	-9.48 a	10.85 d	47.98 a	-13.86 e	18.03 a	44.77 bcd	-13.41 de	17.71 bc
	3	41.75 a	-12.35 bcd	15.41 abc	41.49 efg	-12.58 d	14.58 cde	38.94 d	-12.26 cde	16.54 bc
Span 100 ppm with ultrasound	1	39.36 ab	-12.15 bcd	15.16 abc	40.81 fg	-10.99 b	11.61 fg	43.88 bcd	-10.55 bc	13.58 c
	2	40.99 ab	-14.48 d	18.01 a	43.75 cd	-13.78 e	15.34 cd	42.41 bcd	-12.20 cde	18.82 ab
	3	41.73 a	-13.88 d	16.32 abc	38.40 h	-10.73 b	11.43 fg	39.40 cd	-12.24 cde	17.11 bc

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

Table 2-3. Changes in visual index of tat soi treated with surfactants and ultrasonic wave during storage.

Treatment	No. wash	Visual index on storage time Day 3	Day 9
No-wash	0	9.0 a[z]	8.0 ab
Span 50 ppm	1	9.0 a	8.0 ab
	2	9.3 a	8.3 a
	3	9.0 a	7.7 bc
Span 100 ppm	1	9.3 a	8.0 ab
	2	9.0 a	7.7 bc
	3	9.0 a	8.0 ab
Span 50 ppm with ultrasound	1	9.3 a	8.0 ab
	2	9.0 a	8.0 ab
	3	9.0 a	7.3 cd
Span 100 ppm with ultrasound	1	9.0 a	8.0 ab
	2	9.0 a	7.0 d
	3	9.0 a	7.0 d

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

Table 2-4. Changes in sogginess of tat soi treated with surfactants and ultrasonic wave during storage.

Treatment	No. wash	Sogginess on storage time (%) Day 3	Day 9
No-wash	0	0	0.0 b[z]
Span 50 ppm	1	0	9.5 ab
	2	0	5.3 ab
	3	0	21.3 ab
Span 100 ppm	1	0	11.0 ab
	2	0	15.7 ab
	3	0	16.2 ab
Span 50 ppm with ultrasound	1	0	0.0 b
	2	0	14.2 ab
	3	0	16.7 ab
Span 100 ppm with ultrasound	1	0	8.3 ab
	2	0	22.6 ab
	3	0	43.3 a

[z]Mean within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range teat.

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CONCLUSION

Compare to chlorine (CL) treatment, microbial reduction in electrolyzed water (EW) treatment was significant and effective to reduce microbial populations without a negative impact on seed germination. UVB irrigation for 20 min was effective to reduce the microbial contamination of cultivation tray and other materials used in cultivation. Using the Korea Wonshi nutrient solution enhanced the growth and quality of romaine lettuce and the Yamazaki's nutrient solution for lettuce was most appropriate for enhancing the growth and red color of beet.

Microbial populations of tat soi treated with surfactants and ultrasound were reduced. After 3 days of storage, no significant differences of visual index and sogginess among treatments were found indicating that cleaning with surfactants and ultrasonic waves had a detrimental effect on the quality of baby leaf vegetables.

Fundamental protocols for producing safety baby leaf vegetables in a plant factory were proposed as followed: seed decontamination with EW, cultivation tray sterilization with UVB for 20 min, preharvest washing of baby leaf vegetable 3 times with Span 20 at 50 ppm and ultrasonic waves.

ABSTRACT IN KOREA

본 석사학위 논문은 최근 증가하는 어린잎 채소를 이용하는 즉석편이 식품 소비와 이와 관련한 어린잎 채소 생산 및 유통 과정에서의 미생물관리 정도 향상을 목적으로 수행되었다. 본 연구를 통해 관행의 재배 방법을 고수할 경우 하우스 재배는 물론 식물공장 재배 어린잎 채소에서도 토양 재배 채소와 유사한 수준의 미생물 오염도가 확인되었다. 따라서 이러한 문제점을 해결하기 위하여 여러 가지의 수확전 및 수확후 처리법을 개발하고 그 효과를 검증하였다. 다채, 로메인, 비트를 대상으로 한 모든 실험에서 *E. Coli* 와 *Salmonella* spp. 균은 검출되지 않았다. 각각 5 분, 10 분간 미산성 전해수와 염소수 처리를 한 다채, 로메인, 비트의 종자는 처리하지 않은 무처리구 보다 일반미생물 수가 감소하였다. 염소수 처리구에서는 일반미생물이 가장 많이 감소했으나 발아율이 모두 통계적으로 유의하게 떨어졌다. 반면 미산성 전해수 처리구에서는 적은 염소농도로도 일반미생물 수가 무처리구보다 유의하게 감소하였고, 발아율에는 유의차가 없었다. 또한 재배에 사용되는 재배판을 20 분간 UVB 를 조사하면 일반미생물수를 10% 미만으로 감소시킬 수 있음을 확인하였다. 한국원시, 일본원시, 야마자키 상추용 양액으로 재배하여 어린잎 채소의 품질과 수확량을 높일 수 있었는데

다채와 로메인은 한국원시 양액을, 비트는 야마자키 상추용 양액을 사용하여 재배하는 것이 본연의 색감 유지 및 생육 증대에 유리하였다. 수확한 어린잎 채소에 계면활성제를 처리하면 일반미생물수를 감소시킬 수 있었는데 초음파 처리를 병행하면 그 효과가 유의하게 증대되었다. 그러나, 초음파 세척처리 횟수를 지나치게 증대시키면 장기저장 시 물러짐이 심해지고 감모율이 증대되는 결과가 동반됨을 확인하였다. 본 연구 결과를 통해 식물공장 어린잎 채소의 고품질 안전 생산을 위한 재배 기준 확립과 수확 후 세척 기준에 대한 새로운 방법 및 그 이론적 근거를 마련할 수 있었다.