Protected Horticulture and Plant Factory, Vol. 24, No. 1:45-50, March (2015)
DOI http://dx.doi.org/10.12791/KSBEC.2015.24.1.045
pISSN 2288-0992
eISSN 2288-100X

Growth of and Bacterial Counts on Several Edible Sprouts Exposed to Spray Ionization

Min-Jeong Song[1,2], Yu-Min Jeon[1,2], and Myung-Min Oh[1,2]*

[1]*Division of Animal, Horticultural and Food Science, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Brain Korea Center for Bio-Resource Development, Chungbuk National University, Cheongju 361-763, Korea*

Abstract. Edible sprouts are highly nutritious, containing numerous health-promoting phytochemicals and nutrients. However, the process of sprouting is subject to potential contamination by microorganisms attached to the seed coat. The objective of this study was to determine the effects of air anions generated by spray ionization on growth and sterilization of edible sprouts. Treatment with air anions has positive effects on plant growth and sterilization in 4 kinds of sprouts. Hypocotyl length in red cabbage and kale increased approximately 1.26-fold and radicle length of lettuce, red cabbage, and kale increased 1.4 to 1.6-fold compared to the control. The fresh weight of sprouts in the spray-ionization treatment was significantly (16.0-38.5%) higher than that of the controls. Radicle activity in sprouts exposed to anions was higher than that of the control in all species. Bacterial counts on lettuce, red cabbage, and tatsoi decreased significantly (by 41%, 66%, and 19%, respectively), and bacterial colonies also declined in drainage water. Our findings suggest that spray-ionization treatment is useful for improving sprout growth and for sterilizing sprouts.

Additional key words: air anion, radicle activity, sterilization

Introduction

Sprouts have excellent nutritional and functional value because they contain protein, dietary fiber, minerals, vitamins, and health-promoting phytochemicals that are synthesized during seed germination (Cevallos-Casals and Cisneros-Zevallos, 2010; Waje and Kwon, 2007). Recent research has demonstrated that sprouts contain antioxidant and anticancer properties, which has caused them to receive more attention as healthy, plant-based foods (Gawlik-Dziki et al., 2012; Yuan et al., 2010).

Despite these benefits, sprout consumption is occasionally associated with disease (Alexander and Hammes, 2003). When sprouts are produced using nonsterilized or incompletely sterilized seeds, high humidity and temperature generated by irrigation and seed respiration during germination promote the growth of microorganisms that remain on the seed coat (Peñas et al., 2010; Taormina et al., 1999). At least 40 cases of foodborne disease related to consumption of sprouts are reported to have occurred from 1973 to 2006. *Escherichia coli* O157:H7, *Salmonella* sp., and *Bacillus cereus* were the major bacteria implicated in these diseases, and various treatments (e.g., high pressure, heat, electron-beam and gamma irradiation) have been evaluated for their ability to reduce bacterial growth during postharvest processing of sprouts (Alexander and Hammes, 2003; Cevallos-Casals and Cisneros-Zevallos, 2010; Waje et al., 2009).

Air ions are classified as cations, which gain electrons, and anions, which lose electrons (Kataka, 1978). These molecules are formed by energy generated by colliding neutrons or molecules with elements in soil (e.g., radium and thorium), the radioactive gas radon, ultraviolet light, and cosmic rays. Air anions promote human health by activating cell metabolism and by causing acidic blood to become more alkaline, which confers strong immunity (Chee, 2009). Air anions have been applied in industrial areas because of their sterilizing effects (Yun and Seo, 2013). Two major methods, the high-voltage and spray-ionization methods, are used to generate air anions. The high-voltage method produces negatively charged oxygen molecules by colliding atmospheric oxygen with anions generated by a high-voltage pulse with direct current (Chee, 2009). The spray-ionization method generates oxygen anions by combining electrons from ionized water molecules with oxygen, called the Lenard effect (Park, 2006). A typical example of the Lenard effect in action occurs in waterfalls, where unstable electrons are produced when microdroplets of water strike rock, generating oxygen anions.

*Corresponding author: moh@cbnu.ac.kr
Received March 02, 2015; Revised March 09, 2015; Accepted March 13, 2015

Air anions have been known to have positive effects on plant growth. Exposure of barley to air anions caused a significant increase in height, fresh weight, and dry weight (Krueger et al., 1963b). Growth promotion in plants exposed to excessive air anions has been attributed to increased respiration and photosynthesis, and to the synthesis of cytochromes and iron-binding proteins (Elkiey et al., 1985). To date, research relating to air anions has been limited to a few crops, such as barley and oats (Krueger et al., 1962; Krueger et al., 1963a; Krueger et al., 1965); examination of this effect should be conducted in additional crops, including vegetables such as edible sprouts. The objective of this study was to assess the effects of air anions generated by the spray-ionization method on growth and sterilization of edible sprouts.

Materials and Methods

1. Plant Materials and Growth Conditions

Seeds of four plant species were used in this study: lettuce (*Lactuca sativa*, Asia Seed Co., Ltd., Seoul, Korea); red cabbage (*Brassica oleracea* var. *capitata f. rubra*, Onsem Co., Ltd., Anseong, Korea); tatsoi (*Brassica campestris* var. *narinosa*; Asia Seed Co., Ltd., Seoul, Korea); and kale (*Brassica oleracea* var. *acephala*, Koregon Co., Ltd., Anseong, Korea). Seeds were sown into twelve plastic trays (18 × 9 × 3 cm, L × W × H) in one layer and cultured in two auto-sprouters (EasyGreen Mikro-Farm™, Seed and Grain Technologies, LLC. Albuquerque, USA). The auto-sprouters were placed in a growth chamber equipped with red and blue LEDs (red:blue = 78:22, 85 ± 3 $\mu mol\ m^{-2}\ s^{-1}$, 12-h photoperiod) and the temperature was maintained at 20°C. One auto-sprouter was used as a control and was provided irrigation water by spraying for 15 min every 4 h; the second sprouter was equipped with an ultrasonic fogging system (DH-014, NIT Co. Ltd., Suwon, Korea) for spray-ionization, and was supplied with mist and anions for 37 min every 4 h (Fig. 1). Both treatments supplied same amount of water per day (1.35 L sterile distilled water d^{-1}), sufficient for seed germination. The range of anion concentration applied to the sprouts was 28×10^4~40×10^4 ions cm^{-3} and was measured by an anion counter (COM-3600, Com Systems Ltd., Tokyo, Japan). The range of anion concentration in the control was 27~30 ions cm^{-3}. The trays were rotated daily to minimize differences in light intensity, moisture, and anion concentration according to position.

2. Sprout Growth

Ten sprouts of each species were sampled from the control and spray ionization treatments for measurement of hypocotyl and radicle length, fresh weight, and dry weight at 7 d after seeding. An electronic scale (SI-234, Denver Instruments, Bohemia, USA) was used to determine fresh weight, after which the sprouts were dried at 70°C in an oven (VS-1202D2, Vision Scientific Ltd., Daejeon, Korea) for 72 h to determine dry weight.

3. Radicle Activity

Radicle activity was analyzed in four sprouts of each species prior to cotyledon formation. Samples (about 0.5 g, 10 sprouts/repeat) were placed into 15-mL tubes, and 1% TTC (2,3,5-triphenyl tetrazolium chloride, Sigma-Aldrich, St. Louis, USA) solution, 0.1 M sodium phosphate buffer (pH 7.0), and distilled water were then added (1:4:5 v/v/v), bringing the volume to 10 mL. The tubes were incubated

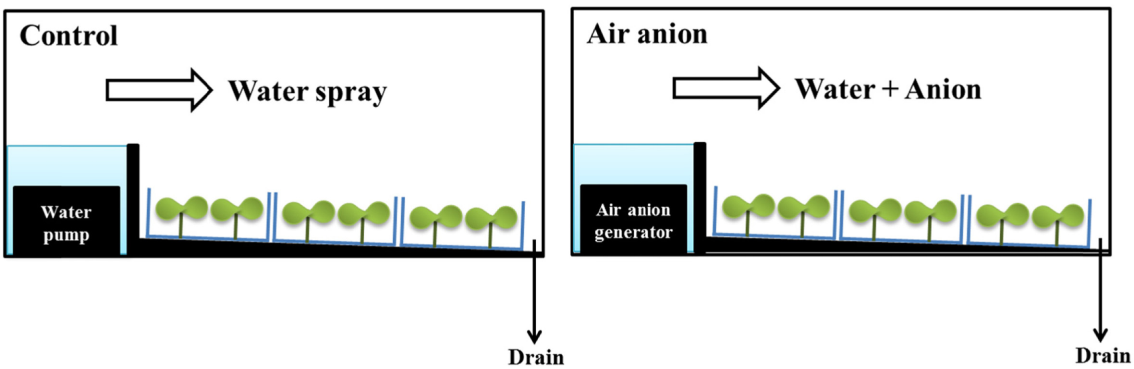

Fig. 1. Two sprout-growing systems for control and air-anion treatment placed on a growth chamber. Sterile distilled water in control was supplied to sprouts by water pump. In air anion treatment, both the distilled water and air anion were applied to sprouts by an air anion generator. Irrigation amount of control and air anion treatment was the same as 1.35 L per day.

for 4 h at 37°C in a water bath (MSB-2011D; Mono-tech Eng. Co. Ltd., Siheung, Korea) in the dark. The reaction was then stopped by adding 3 mL of 2 N H_2SO_4. The solutions were passed through a filter paper (No. 2; Whatman International Ltd., Maidstone, UK); radicles remaining on the filter paper were dried with gauze and ground with a mortar and pestle, and 10 mL ethyl acetate and sea sand were added to extract formazan. The supernatant containing extracted formazan was collected, and optical density was measured at 470 nm using a spectrophotometer (UV-1800, Shimadzu Co. Ltd., Kyoto, Japan). A standard curve was prepared using 1,3,5-triphenyltetrazolium formazan (TPF, Sigma-Aldrich, St. Louis, USA) standard solution (0, 2.5, 5, 10, 20, 40 ppm) dissolved in ethyl acetate. Radicle activity was expressed by mg formazan generated h^{-1} g $radicle^{-1}$.

4. Bacterial Counts

Test solutions were prepared by homogenizing sprouts (about 5 g) with 50 mL sterile distilled water; drainage water from the auto-sprouters was sampled for bacterial counts at 7 d after seeding. One milliliter of test solution was diluted with sterile distilled water in a series of 10 dilutions. The diluted test solution (50 μL) was smeared on nutrient agar medium (Difco™, Becton, Dickinson and Company, Franklin Lakes, USA) and incubated at 28°C for 2 d. Bacterial colonies were counted and presented as the product of the number of colonies and number of dilutions.

5. Statistical Analysis

This experiment was replicated twice to verify reproducibility. Analysis of variance (ANOVA) was performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC). Means among treatments were compared using Student's t-test.

Results and Discussion

1. Growth

Hypocotyl and radicle length were influenced by spray ionization in each of the four tested species (Fig. 2). Hypocotyl length increased significantly in red cabbage and kale sprouts exposed to air anions, by 26% and 27% respectively, compared with the control; hypocotyl length of lettuce and tatsoi sprouts did not differ between the control and anion treatments. Radicle length in lettuce, red cabbage, and kale sprouts subjected to spray-ionization was 60%, 64%, and 45% higher than that of the control, respectively, while radicle length of tatsoi did not differ significantly.

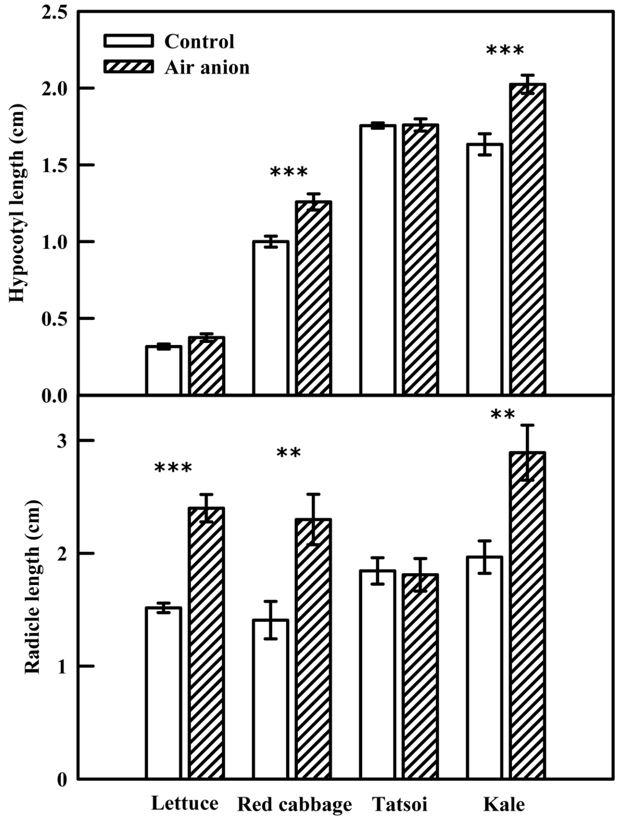

Fig. 2. Hypocotyl and radicle length of various edible sprouts exposed to air anion at 7 days after seeding. The concentration of air anion treated to sprouts was 28 × 104~40 × 104 ion·cm^{-3}. The data are the means and the bars indicate standard errors (n=10). Significant at **P = 0.01 and ***P = 0.001.

Air-anion treatment had significant positive effects on sprout FW and DW (Fig. 3). The FW of lettuce, red cabbage, tatsoi, and kale sprouts exposed to spray-ionization was 38.5%, 34.3%, 25.3%, and 16.0% higher than that of the control, respectively. Dry weights of lettuce, tatsoi, and kale sprouts in the air-anion treatment were significantly higher than DW of the respective control treatments. Hypocotyl and radicle lengths of tatsoi sprouts were not influenced by air-anion treatment, but hypocotyl diameter was large, causing apparent increases in FW and DW (data not shown). Spray ionization generally had a positive effect on seed germination and sprout growth, although the growth response differed among the species. Lim et al. (2011) reported that germination and growth rate of brown rice were improved by air-anion treatment. A previous study also reported that air anions improved lettuce growth (Song et al., 2014; Wachter and Widmer, 1976). Air anions may stimulate respiration in seeds by promoting oxygen uptake (Krueger et al., 1963b). Since we controlled for environmental factors, including moisture condition in relation to seed germination, our findings may be a result of promotion of seed respiration by air anions.

2. Radicle Activity

Spray-ionization treatment increased radicle activity prior to cotyledon generation in all sprout species (Fig. 4). Tatsoi showed the highest rate of increase (42.6%), followed by red cabbage (21.7%), lettuce (19.0%), and kale (17.2%). When colorless tetrazolium combines with hydrogen ions generated by dehydrogenase, tetrazolium is converted to formazan (red), with the quantity of red representing the level of radicle activity (Jones and Prasad, 1969). Thus, the increased radicle activity observed here may have represented increased respiration due to vigorous dehydrogenase activity suggesting that spray-ionization may stimulate respiration of seeds during germination.

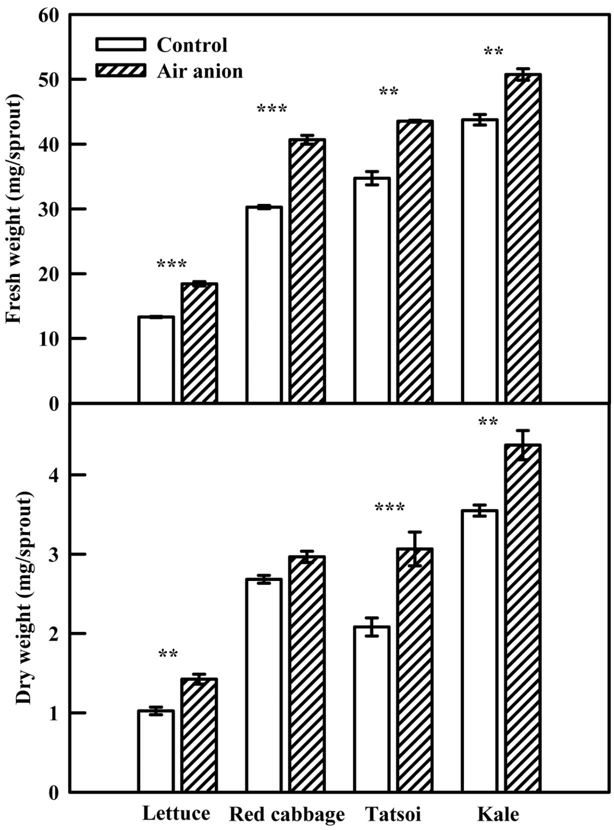

Fig. 3. Fresh and dry weight of various edible sprouts exposed to air anion at 7 days after seeding. The concentration of air anion treated to sprouts was 28×10^4~40×10^4 ion·cm^{-3}. The data are the means and the bars indicate standard errors (n = 10). Significant at $^{**}P = 0.01$ and $^{***}P = 0.001$.

3. Bacterial Counts

Spray-ionization of sprouts had a sterilizing effect (Fig. 5). With the exception of kale, the number of bacterial col-

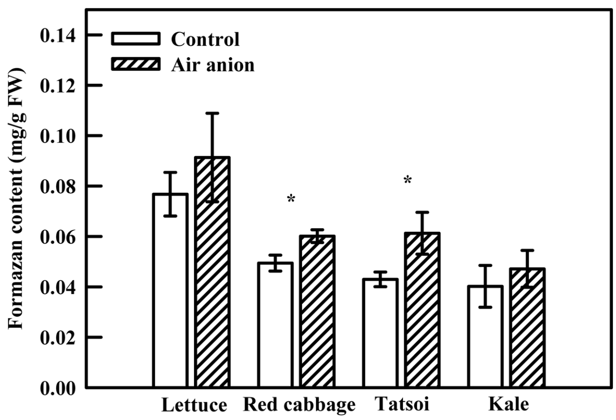

Fig. 4. Radicle activity of various edible sprouts exposed to air anion before emerging cotyledon. The concentration of air anion treated to sprouts was 28×10^4~40×10^4 ion·cm^{-3}. The data are the means and the bars indicate standard errors (n = 4). Significant at $^{*}P = 0.05$.

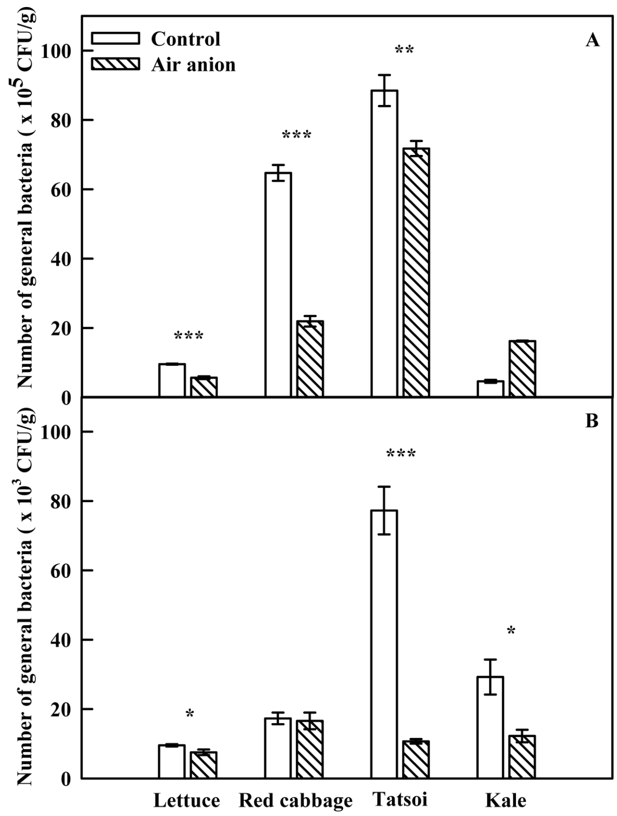

Fig. 5. Bacterial count of various edible sprouts (A) and drained water (B) exposed to air anion at 7 days after seeding. The concentration of air anion treated to sprouts was 28×10^4~40×10^4 ion·cm^{-3}. The data are the means and the bars indicate standard errors (n = 3). Significant at* $P = 0.05$, $^{**}P = 0.01$ and $^{***}P = 0.001$.

onies associated with each sprout species in the anion treatment was lower than that in the control at 7 d after seeding. Anion treatment caused a significant (66.1%) reduction in bacterial counts in red cabbage sprouts. Bacterial colonies in spray-ionization-treated lettuce and tatsoi sprouts were significantly reduced, by 40.8% and 18.9%, respectively. Numbers of colonies were reduced by 78%, 11%, 36% in the drainage water of lettuce, tatsoi, and kale, respectively. These results suggest that air anions suppressed the propagation of bacteria on seed coats. Arnold et al. (2004) reported that outbreaks of bacterial pathogens such as *Campylobacter jejuni*, *E. coli*, *Salmonella enteritidis*, and *Listeria monocytogenes*, and spores of *Bacillus stearothermophilus* were nearly eliminated by exposure to air anions. A sterilization effect of air anions was also observed in *Candida albicans* (Sharagawi et al., 1999). Kellogg et al. (1979) reported that superoxide radicals generated by air anions enhanced the surface charge of *Staphylococcus albus*, causing de-esterification that weakened bacterial cell membranes and resulted in cell death. The effects of spray ionization on sterilization can be interpreted similarly in the present study. Sprouts can be produced more safely by applying spray ionization, which reduces the threat of infection by bacteria such as *E. coli* O157:H7, *Salmonella* sp., and *Bacillus cereus*, which are commonly present on edible sprouts.

Conclusion

Spray-ionization treatment enhanced the growth of edible sprouts of four plant species by increasing respiration, although sensitivity to air-anion treatment differed among the species. Anion treatment also demonstrated a sterilization effect, reducing the generation of bacteria that present major drawbacks to sprout production. Incorporation of air-anion technology into sprout production should provide positive effects on yield and safety.

Acknowledgement

This work was supported by the research grant of Chungbuk National University in 2012.

Literature Cited

Alexander, W. and W.P. Hammes. 2003. Thermal seed treatment to improve the food safety status of sprouts. J. Appl. Bot. 77:152-155.

Arnold, J.W., D.H. Boothe, and B.W. Mitchell. 2004. Use of negative air ionization for reducing bacterial pathogens and spore on stainless steel surfaces. Poulty Sci. 13:200-206.

Cevallos-Casals, B.A. and L. Cisneros-Zevallos. 2010. Impact of germination on phenolic content and antioxidant activity of 13 edible species. Food Chem. 119:1485-1490.

Chee CK, Effect of air anion. Living books. 2nd ed, Korea (2009).

Elkiey, T.M., S. Bhartendu, and N. Barthakur. 1985. Air ion effect on respiration and photosynthesis of barley and Antirrhinum majus. Intl. J. Biometeorol. 29:285-292.

Gawlik-Dziki, U., M. Jezyna, M. Swieca, D. Dziki, B. Baraniak, and J. Czyz. 2012. Effect of bioaccessibility of phenolic compounds on in vitro anticancer activity of broccoli sprouts. Food Res. Intl. 49:469-476.

Jones, P.H. and D. Prasad. 1969. The use of tetrazolium salts as a measure of sludge activity. J. Water Pollution Control Federation 41:441-449.

Kellogg, E.W., M.G. Yost, N. Barthakur, and A.P. Krueger. 1979. Superoxide involvement in the bactericidal effects of negative air ions on Staphylococcus albus. Nature 281:400-401.

Kotaka, S. 1978. Effects of air ions on microorganisms and other biological materials. Critical Rev. Microbiol. 6:109-149.

Krueger, A.P., S. Kotaka, and C.P. Andriese. 1962. Studies on the effects of gaseous ions on plant growth. The influence of positive and negative air ions on the growth of Avena sativa. J. Gen. Physiol. 45:879-895.

Krueger, A.P., S. Kotaka, and C.P. Andriese. 1963a. A study of the mechanism of air-ion-induced growth stimulation in Hordeum vulgaris. Intl. J. Biometeorol. 7:17-25.

Krueger, A.P., S. Kotaka, and C.P. Andriese. 1963b. Gaseous-ion-induced stimulation of cytochrome c biosynthesis. Nature 200:707-708.

Krueger, A.P., S. Kotaka, and C.P. Andriese. 1965. Air ion effects on the oxygen consumption of barley seedlings. Nature 208:1112-1113.

Lim, K.T., J.H. Kim, and J.H. Chung. 2011. Development of an automatic brown rice germinating system of air-phase type with intermittent water spraying and anion radiation. J. Biosystems Eng. 36:187-194.

Park, B.S. 2006. Characteristics of negative ions generated by Lenard effect. J. Natural Sci. 5:38-43.

Peñas, E., R. Gómez, J. Frías, and C. Vidal-Valverde. 2010. Effects of combined treatments of high pressure, temperature and antimicrobial products on germination of mung bean seeds and microbial quality of sprouts. Food Control 21:82-88.

Shargawi, J.M., E.D. Theaker, D.B. Drucker, T. MacFarlane, and A.J. Duxbury. 1999. Sensitivity of Candida albicans to negative air ion streams. J. Appl. Microbiol. 87:889-897.

Song, M.-J., T.-H. Kang, C.-S. Han, and M.-M. Oh. 2014. Air anions enhance lettuce growth in plant factories. Hort. Environ. Biotechnol. 55:293-298.

Taormina, P.J., L.R. Beuchat, and L. Slutsker. 1999. Infections associated with eating seed sprouts: an international concern. Emerging Infectious Diseases 5:626-634.

Wachter, S.L. and R.E. Widmer. 1976. The effects of negative air ions on plant growth. HortScience 11:576-578.

Waje, C.K. and J.H. Kwon. 2007. Improving the food safety of seed sprouts through irradiation treatment. Food Sci. Biotechnol. 16:171-176.

Waje, C.K., S.Y. Jun, Y.K. Lee, B.N. Kim, D.H. Han, C. Jo, and J.H. Kwon. 2009. Microbial quality assessment and pathogen inactivation by electron beam and gamma irradiation of commercial seed sprouts. Food Control 20:200-204.

Yuan, G., X. Wang, R. Guo, and Q. Wang. 2010. Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. Food Chem. 121:1014-1019.

Yun, S.J. and Y.J. Seo. 2013. Removal of bacteria and odor gas by an alumina support catalyst and negative air ions. J. Aerosol Sci. 58:33-40.

음이온 처리된 몇 가지 새싹채소의 생장과 세균 수

송민정[1,2] · 전유민[1,2] · 오명민[1,2]

[1]충북대학교 축산 · 원예 · 식품공학부

[2]BK21 플러스 생물건강소재산업화 사업단

새싹은 영양분이 풍부한 식품이다. 하지만 생산 과정에서 종피에 있는 미생물에 의한 오염 가능성이 존재한다. 이 실험의 목적은 음이온 처리가 새싹의 생장과 살균에 미치는 효과를 구명하는 것이다. 음이온 처리는 4종류의 새싹의 생장과 살균효과에 긍정적인 효과를 보였다. 대조구에 비해 음이온 처리한 적양배추와 케일 새싹의 배축길이는 약 1.26배 증가하였으며, 상추, 적양배추, 케일 유근의 길이는 1.4~1.6배 증가하였다. 모든 새싹의 생체중은 음이온 처리했을 때 대조구에 비해 16.0~38.5% 유의적으로 증가하였다. 유근의 활력 또한 음이온 처리가 대조구에 비해 유의적으로 높은 수치를 보였다. 음이온 처리된 상추, 적양배추, 다채 새싹의 일반 세균 수는 대조구에 비해 각각 41%, 66%, 19% 감소하였으며, 배수되는 물의 세균 수 또한 감소되었다. 결국 음이온처리는 새싹의 생장을 향상시켰으며, 동시에 살균하는데도 효과적이었다.

추가 주제어: 공기음이온, 유근 활력, 살균