ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 108, No. 1 (2021), p. 87–94
DOI 10.13080/z-a.2021.108.012

Surface sterilisation using chemical or physical methods influence microbial growth and quality of green asparagus

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Abstract

In the present study, the different effects of surface disinfectants and sterilisation methods on the antimicrobial efficacy and preserving quality of green asparagus (*Asparagus officinalis* L.) during cold storage were evaluated, both initially after sterilisation pre-treatment and after 21 days of storage. Hot water (H_2O), sodium hypochlorite (NaClO) solution, hot NaClO solution, chlorine dioxide (ClO_2), ozone (O_3) and UV-C exposure were used for surface sterilisation with no pre-treatment as a control group. The visual quality, physiological and biochemical characteristics, and the correlation between sterilisation pre-treatments and green asparagus quality were analysed. In this research, all tested disinfectants and sterilisation pre-treatments were more effective in reducing the microbial load than the control group. The O_3, ClO_2 and NaClO pre-treatments showed remarkable effectiveness in inhibiting the growth of bacteria (especially *Escherichia coli*), yeast and mould with a lower number of microorganisms after 21 days of cold storage. Meantime, hot water washing was better to preserve the nutritional and visual quality and led to minimal damage to green asparagus with higher soluble solids content (SSC) and hue angle values and lower electrolyte leakage (EL). No significant influence was observed on firmness and gas conditions in any of the packages. Significant correlation was present between sterilisation pre-treatments and SSC and colour. Although all pre-treatments effectively inhibited the development of microorganisms and significantly extended the shelf life of green asparagus, the O_3, ClO_2 and NaClO pre-treatments are more worth consideration.

Key words: chlorine dioxide gas, hot water, ozone gas, sodium hypochlorite solution, UV-C.

Introduction

Green asparagus (*Asparagus officinalis* L.) has met with increasing concern and market demand for proper nutrition, medicinal and health value. Green asparagus not only undergoes a series of considerable physiological changes, including water and some nutrition loss due to high respiration rate, but also can be infected by microorganisms after harvest (Huyskens-Keil, Herppich, 2013).

Thrips (Thysanoptera) are severe pests of horticultural crops pre- and post-harvest, usually carrying microorganisms and viruses (Miyata et al., 2016), leading to the deterioration of quality. The primary spoilage microbes in fresh horticultural crops include yeasts and moulds, and total aerobic bacteria (Kuorwel et al., 2013), and as time goes on, their numbers increase. In addition to spoilage microorganisms, the food-borne pathogens are also severe such as some *Escherichia coli*, which can cause urinary tract infections or diarrhoeal diseases (Berg, 2004).

It has been reported that nearly 30% of the post-harvest losses are unfit for consumption due to the spoilage (Yahaya, Mardiyya, 2019). To preserve quality and increase the shelf life of horticultural crops and to inhibit the growth of pathogens, post-harvest treatments are usually essential, and different chemical fungicides and surface disinfectants or physical sterilisation methods have been widely applied to fruits and vegetables. Washing and immersing have been confirmed to be useful in decreasing the initial levels and activities of microorganisms and pathogens. Water or the addition of citric acid or sodium hypochlorite has been widely used to reduce microbial loads and remove debris and cellular fluid (Chen et al., 2016). Chlorine (Cl) is one of the most common sanitisers used at a concentration of 100 mg L^{-1} for fresh vegetables. It has been found that the microbial load decreases after treatment with Cl, which is most used by the fresh-cut industry due to its antimicrobial activity and low cost (Islam et al., 2017).

Please use the following format when citing the article:
Wang L.-X., Choi I.-L., Kang H.-M. 2021. Surface sterilisation using chemical or physical methods influence microbial growth and quality of green asparagus. Zemdirbyste-Agriculture, 108 (1): 87–94. DOI 10.13080/z-a.2021.108.012

The effects of ultrasound sanitisation technology and ozone on fruits and vegetables have also been discussed (São José et al., 2014). Ozone as a surface disinfectant has a high oxidising activity to ensure the microbiological safety and low residues in food (Tiwari et al., 2010). Moreover, essential oils, edible coatings, controlled atmosphere storage and modified atmosphere packaging have been shown to maintain the quality and extend shelf-life (Shao et al., 2015).

Therefore, sterilisation pre-treatment is a vital step for post-harvest vegetables to maintain quality and prolong shelf-life. Antimicrobial activity and a negligible effect on the sensory quality of the product are essential for sterilisation. Therefore, the sensory quality and growth of microorganisms should be evaluated after sterilisation treatments. Because of the rapid growth of the remaining microorganisms, the maintenance of this sterilisation efficiency is essential; as is the initial microbial reductions on the sterilisation day zero (Ragaert et al., 2007).

Consequently, the objective of this research was to evaluate the efficiencies of different chemical and physical sterilisation methods, including hot water (H_2O), sodium hypochlorite (NaClO) solution, hot NaClO solution, chlorine dioxide (ClO_2, gas), ozone (O_3, gas) and UV-C exposure in preventing microorganism growth (total aerobic bacteria, yeast and mould and *E. coli*). Besides, gas conditions (CO_2, O_2 and C_2H_4), physiological and biochemical qualities such as fresh weight loss, soluble solids content (SSC) and electrolyte leakage (EL) as well as sensory characteristics were determined during cold storage.

Materials and methods

Plant material. Green asparagus (*Asparagus officinalis* L., cultivar 'Welcome') was cultivated and provided by a local farm in Yanggu-gun (lat. 38°12′33.00″ N, long. 127°13′3.00″ E), Gangwon-do, Republic of Korea on 20 May 2019. The fresh asparagus spears were immediately transported to Post-harvest Physiology and Distribution Laboratory of Kangwon National University. Healthy and uniform (1.4 ± 0.1 cm diameter and 24 ± 1.0 cm length) green asparagus spears were randomised and placed at 4 ± 0.5°C temperature until treated for this study.

Sterilisation pre-treatments and packaging. Whole green asparagus spears were pre-treated as in Table 1.

Table 1. The sterilisation pre-treatments application conditions of green asparagus spears

No.	Pre-treatment	Application conditions
1.	Control	no sterilisation
2.	Hot H_2O	immersed in 48 ± 0.5°C hot water for 2 min
3.	NaClO	immersed in 150 ppm sodium hypochlorite solution for 15 min
4.	Hot NaClO	immersed in 48 ± 0.5°C 150 ppm hot NaClO solution for 2 min
5.	ClO_2	exposed in 4 ± 0.5°C refrigerator filled with 5 ppm chlorine dioxide gas for 30 min
6.	O_3	exposed in 4 ± 0.5°C refrigerator filled with 5 ppm ozone gas for 60 min
7.	UV-C	irradiated with 15 kJ m^{-2} UV-C exposure for 5 min

The green asparagus was then packaged with a 10,000-cc m^{-2} day^{-1} atm^{-1} oxygen transmission rate (Dae Ryung Precision Packaging Industry Co. Ltd., Korea). All groups were stored at 4 ± 0.5°C temperature and 85% humidity until the final day. Each treatment was replicated four times.

Microbiological analysis. Microbiological analysis was performed on green asparagus immediately after sterilisation pre-treatments and after 21 days of cold storage according to Choi et al. (2015) method with some modifications. Approximately 2.0 g of fresh asparagus was mixed with 18 ml of diluent (sterilised water) using a stomacher (Powermixer, B&F Korea) set at the highest speed (level 10, 200 rpm) for 3 min. The mixture was then diluted to the 1:1000, and 1.0 ml dilution was dropped on the Petrifilm plates and cultivated for 24–72 h. The growth of total aerobic bacteria, yeast and mould, and *Escherichia coli* was calculated using 3M Petrifilm aerobic count plates (3M Co., USA). The number of microorganisms was represented by base 10 logarithm of the colony-forming unit (log CFU g^{-1}) concentration.

Atmosphere analysis. Gas samples were taken from the packages. Carbon dioxide (CO_2) and oxygen (O_2) concentrations in the different treatments were measured with an infrared CO_2/O_2 analyser Check Mate 9900 (PBI-Dansensor, Denmark); ethylene (C_2H_4) concentrations were measured using a GC-2010 Shimadzu Gas Chromatography GC-2010 (Shimadzu Co., Japan) (Yoon et al., 2018). The changes in atmospheric composition inside the packages were measured every 5 days during the entire cold storage period (21 days).

Sensory quality evaluation. The general acceptability of asparagus spears was assessed by five skilled panellists to determine the overall appearance of asparagus, including visual quality (VQ) and impairment during storage and after 21 days of cold storage according to the Choi et al. (2015) method. Asparagus VQ was assessed on a scale of 5 to 1 (5 – best, 4 – better, 3 – good, 2 – bad, 1 – worst), and off-odour was assessed on a scale of 0 to 5 (0 – no off-odour, 5 – extremely strong off-odour). Asparagus spears with a VQ score equal to or greater than 3 and an off-odour score of less than 3 were determined to be suitable for sale.

Physicochemical and biochemical traits. Fresh weight loss rates were measured on 1, 3, 5, 10, 15 and 21 day from the first day according to the following formula:

Fresh weight (FW) loss rate = (initial fresh weight − final fresh weight) / initial fresh weight × 100%.

Firmness was measured at two locations on each asparagus spear from tips (from top-most 5 cm) and stems (from lower-most 8 cm) using a rheometer Compac-100 (Sun Scientific Co. Led., Japan) with a probe (Ø 3.0 mm) at 1.0 mm sec^{-1} speed on the initial day and after 21 days of cold storage.

The colour parameters of asparagus spears, including hue angle (hue angle = arctan (b*/a*)), a* (A negative value of a* indicates green, while a positive number indicates red), b* (positive b* indicates yellow and negative indicated blue) and L* (lightness, 0 = black and 100 = white) were measured on the initial day and after 21 days of cold storage following Yoon et al. (2017) using a colour-difference meter CR-400 (Konica Minolta Sensing Inc., Japan). The total colour difference (ΔE^*) was represented and calculated according to Zhang et al. (2020):

$$\Delta E^* = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}.$$

Soluble solids content (SSC) on the initial day and after 21 days of cold storage was measured following the protocol described by Yoon et al. (2017). The middle part of asparagus spears was chopped to measure SSC using a pocket refractometer PAL-1 (Atago, Japan) and indicated as °Brix.

To identify the conductivity, which indicated the level of tissue damage, electrolyte leakage (EL) on the initial day and after 21 days of cold storage was measured following Yoon et al. (2018). Approximately 0.6 g of fresh asparagus slices were excised from asparagus spears, placed in 25 ml of 0.4 M mannitol and incubated for 3 h at ambient temperature. The EL was immediately measured after sterilisation pre-treatment using electrical conductivity (EC) meter Cyberscan PC 300 (Eutech Instruments, Singapore). The EL was also determined on the same sample after being frozen at −20°C temperature for 24 h and then thawed. The EL was determined on the initial sterilisation day and after 21 days of cold storage and calculated using the following equation:

Relative EL = initial EL / finial EL × 100%.

Statistical analysis. The software of *Microsoft Excel* and *IBM SPSS Statistics*, version 24 (IBM Corp., USA) was used to determine the statistical characteristics of the data. Significant differences were tested with ANOVA (one-way analysis of variance) and Duncan's multiple comparisons test at the $P < 0.05$ level. The standard error (SE) of each mean is presented (n = 5).

Results and discussion

Microbiological analysis. Immediately after sterilisation pre-treatment and after 21 days of cold storage, the number of microorganisms, including total aerobic bacteria, yeast and mould and *Escherichia coli*, were counted. According to Table 2, the green asparagus showed initial contamination values of 4.10 log CFU g^{-1} of total aerobic bacteria, 1.43 log CFU g^{-1} of yeast and mould and 1.49 log CFU g^{-1} of *E. coli*. After sterilisation pre-treatments, the number of microorganisms decreased, though they had increased after 21 days of cold storage.

Table 2. The effects of chemical and physical sterilisation pre-treatments on the growth of microorganisms on the initial day and after 21 days of cold storage

Pre-treatment	Number of microorganisms, log CFU g^{-1}					
	total aerobic bacteria		yeast and mould		*Escherichia coli*	
	initial day	after 21 days	initial day	after 21 days	initial day	after 21 days
Control	4.10 a	5.57 cd	1.43 a	3.10 a	1.49 ab	4.90 a
Hot H_2O	3.90 b	5.81 b	0.00 b	2.33 a	2.48 a	3.85 c
NaClO	3.66 c	5.90 a	0.00 b	0.67 a	2.46 a	3.67 c
Hot NaClO	3.62 c	5.81 b	0.00 b	2.10 a	0.00 c	4.02 bc
ClO_2	3.75 bc	5.62 c	0.00 b	0.67 a	0.67 bc	4.40 ab
O_3	3.15 d	5.23 e	0.00 b	1.00 a	0.00 c	4.97 a
UV-C	3.27 d	5.51 d	0.77 ab	2.16 a	0.00 c	4.51 ab

Note. Explanation of pre-treatments in Table 1; means (n = 5) followed by the same letter in the column are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

In particular, the O_3 pre-treatment showed the highest inhibition with the lowest number of total aerobic bacteria both on the initial day and after 21 days of cold storage, followed by the UV-C pre-treatment. The O_3 as a kind of antioxidant can result in toxicity to microorganisms due to the progressive oxidation of phospholipids and proteins in microbial cell membranes (García-Martín et al., 2018). The efficiency of UV-C on microorganism inhibition has also long been elucidated (Jeon, Ha, 2018). For yeast and mould subjected to different conditions, except for the control and UV-C pre-treatments with 1.43 and 0.77 log CFU g^{-1}, all other groups obtained 0 log CFU g^{-1} immediately after sterilisation pre-treatment. While no significant difference was observed, the NaClO and ClO_2 pre-treatments showed lower average values of approximately 0.67 log CFU g^{-1}, followed by O_3 with 1.0 log CFU g^{-1} after 21 days of cold storage. The *E. coli* microbial population was 0 log CFU g^{-1} after the hot NaClO, O_3 and UV-C pre-treatments, but higher microorganism numbers were observed in the control, hot H_2O and NaClO pre-treatments, subsequently reaching 3.5–5.0 log CFU g^{-1} in all groups after 21 days of cold storage.

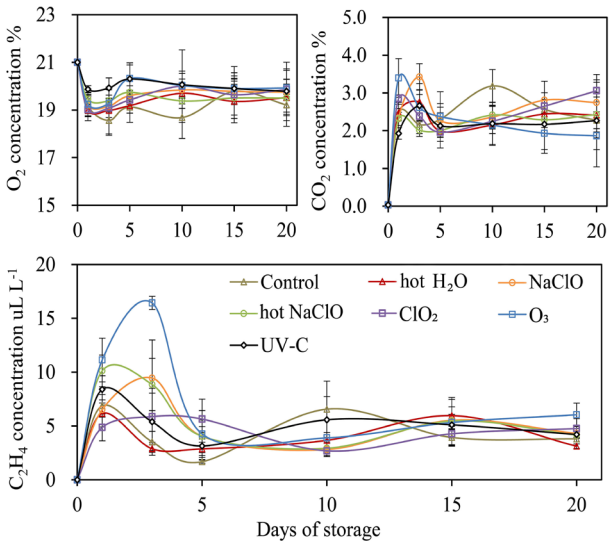
The persistent antiseptic effects of the hot NaClO, O_3 and UV-C pre-treatments were not significant in *E. coli*. The NaClO, hot NaClO and ClO_2 pre-treatments showed similar sterilisation efficiencies immediately after sterilisation pre-treatments, considering harmful by-products and residual moisture; ClO_2 could be used as an alternative to aqueous sanitisers such as chlorine and NaClO solutions (Keskinen et al., 2009).

Different sensitivities of microorganisms to the different kinds of chemical and physical sterilisation methods were exhibited. Considering all microbial populations, the O_3 and UV-C pre-treatments exhibited higher sterilisation efficiencies after the initial day of pre-treatment. Similarly, the O_3 and NaClO pre-treatments showed more potent inhibition to all microorganisms after 21 days of cold storage. The strong effectiveness of O_3 for many plants has been confirmed, which was, to some extent, attributed to the high oxidation potential (Miller et al., 2013). In conclusion, the O_3 pre-treatment was more beneficial during cold storage of green asparagus.

Atmosphere analysis. During green asparagus cold storage, the gas conditions in modified atmosphere (MA) packages, including CO_2, O_2 and C_2H_4 concentrations, were elevated (Fig. 1).

Trends of gas content were similar among the different pre-treatments, increasing rapidly and then decreasing and remaining stable during cold storage, but with different thresholds. The gas change was related to the respiration rate, which provides energy for vegetables and fruits, and the gas condition in MA packages significantly affected the respiration during post-harvest storage. Physiological stress caused by damage or processing increased the respiration rate of the produce, and high respiration rates and/or lower energy reserves led to shorter shelf-life (Eskin, 1990).

The CO_2 concentration increased in the first three days of cold storage and reached the highest level. The CO_2 concentrations in the O_3 and NaClO pre-treatments



Note. Vertical bars represent the mean ± SE (n = 5).

Figure 1. Changes in oxygen (O_2), carbon dioxide (CO_2) and ethylene (C_2H_4) concentrations in modified atmosphere (MA) packages of green asparagus after 21 days at cold storage

were higher than those of the other sterilisation pre-treatments and the control group, where it was nearly 3.5%. Subsequently, all groups slightly decreased and remained stable at 2–3.5%. The CO_2 concentration of the hot H_2O pre-treatment increased gradually between the 5th and 10th days of cold storage and then decreased after the 10th day. The O_2 concentration with all pre-treatments slightly decreased on the first day of storage but remained above 18.0% at later storage time points. An O_2 concentration of 3% or lower can lead to the deterioration caused by anaerobic respiration. Thus, the modified atmosphere conditions were suitable for cold storage of green asparagus with CO_2 concentration of 5–12% and O_2 concentration higher than 5% (Kader, 2002). The effect of sterilisation pre-treatments on the respiration rate has been demonstrated in numerous experimental trials. The O_3 and precooling showed better retention effect in respiration intensity in mulberry (Han et al., 2017), but the respiration rate decreased under the ClO_2 pre-treatment in litchi fruit (Wu et al., 2011).

The trend of C_2H_4 concentration change was similar to that of the CO_2, which declined rapidly after reaching the maximum value in the first three days of cold storage and remained largely stable after the 10th day, nearly 5 µL L^{-1}. The sterilisation pre-treatments increased the concentration of C_2H_4 during subsequent cold storage, possibly caused by the physical stress that related to the endogenous C_2H_4 synthesised from the physical stress of green asparagus, which is consistent with the hot water and peeled treatment of white asparagus (Siomos et al., 2010). The transient C_2H_4 production induced by the O_3 pre-treatment was higher than that in other pre-treatments but decreased and remained at a lower level. Moreover, O_3 with the antioxidant capacity can reduce the levels of some harmful substances such as C_2H_4 (Xu et al., 2019). The changes in CO_2, O_2 and C_2H_4 concentrations in MA packages were not very different between the control and sterilisation pre-treatments, likely indicating that not all pre-treatments in this research cause serious stress to green asparagus. In conclusion, as acceptable abiotic stress, all sterilisation pre-treatments could inhibit the growth of microorganisms and retard the decrease in quality.

Physicochemical and biochemical traits. The soluble solid content (SSC), firmness, hue angle and electrolyte leakage (EL) of green asparagus with different sterilisation pre-treatments are shown in Table 3. The acceptability of green asparagus to some extent depends on the taste, colour and firmness.

Table 3. Changes in physicochemical and biochemical traits of green asparagus immediately after sterilisation pre-treatments and after 21 days of cold storage

Pre-treatment	SSC °Brix	Firmness kg		Hue angle °		Total colour difference (ΔE^*)		Electrolyte leakage (EL)	
		tip	stem	tip	stem	tip	stem	0 day[1]	finial
Initial	6.40 a	1.31 ab	1.28 ab	121.7 a	112.0 a	0.00	0.00	–	–
Control	4.10 cd	1.35 ab	1.40 ab	120.9 abc	115.5 e	18.96 a	10.39 a	0.43 b	0.39 ab
Hot H_2O	4.45 b	1.27 b	1.27 b	121.2 ab	118.8 ab	13.43 ab	8.60 a	0.65 ab	0.16 c
NaClO	4.28 bc	1.28 ab	1.34 ab	120.6 abc	118.4 bc	11.86 b	9.53 a	0.67 ab	0.22 c
Hot NaClO	3.75 e	1.32 ab	1.22 ab	121.2 a	117.0 cde	14.47 ab	10.06 a	0.66 ab	0.29 bc
ClO_2	4.30 bc	1.42 a	1.22 a	119.7 bc	117.1 cde	15.89 ab	10.21 a	0.57 ab	0.22 c
O_3	4.18 cd	1.27 b	1.26 b	118.8 c	117.4 bcd	16.81 ab	10.23 a	0.82 a	0.46 a
UV-C	3.98 d	1.30 ab	1.24 ab	120.0 abc	116.2 de	17.40 ab	10.47 a	0.69 ab	0.21c

Note. Explanation of pre-treatments in Table 1; [1] – immediately after the sterilisation pre-treatment; values are the mean of 5 samples; means (n = 5) followed by the same letter in the column are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

SSC not only indicates the nutrition but is also related to the firmness of fruits and vegetables and directly affects the taste. Compared to the initial day following sterilisation, the SSC decreased from 6.40 to approximately 4.0. Among all pre-treatments, the hot H_2O pre-treatment was best at retarding the decrease in SSC, and the hot NaClO pre-treatment had the lowest SSC of 3.75, corresponding to a loss of approximately 41.4% of the initial amount. The UV-C pre-treatment at 6.0 kJ m^{-2} inhibited the increase of the soluble solids content and titratable acidity ratio (SSC:TA) during blueberry fruit post-harvest storage (Nguyen et al., 2014), which was similar to the lower SSC observed in this study. The ClO_2 and O_3 pre-treatments also exhibited higher SSC than the control, the hot NaClO and UV-C pre-treatments, in agreement with the results that the gaseous phase of O_3 maintained higher SSC likely due to the inhibition of the respiratory response (Chen, Zhu, 2011), and the ClO_2 pre-treatment was more beneficial for total SSC in strawberry (Aday, Caner, 2014). The high physiological activity and respiration rate possibly resulted in the fast hydrolysis of soluble solids (Zhang et al., 2007), the effectiveness of chemical sanitisers could slow down the metabolic activity, and the sterilization efficiency of chemical sanitisers is enhanced by the cavitation activity of ultrasound (Maslak, Weuster-Botz, 2011; Aday, Caner, 2014).

Firmness, which is an important factor for assessing asparagus quality, was measured on the last storage day. Textural changes, including increases in toughness and fibrousness due to the development of fibre and the lignification (Herppich, Huyskens-Keil, 2008), decreases in the galactose and arabinose contents known as cell wall polysaccharide decreased, while xylose and the warm alkali-soluble lignin content increased during shelf-life of asparagus (Everson et al., 1992). Increases in firmness were detected for the control, hot NaClO and ClO_2 pre-treatments in the top portion (tip), while the firmness in the control and NaClO pre-treatments was higher than the initial values in stems, but the difference was not significant both in tips and in stems. This increase is related to the development of lignin and the activity of phenylalanine-ammonia-lyase (PAL) (Lipton, 1990). The firmness data obtained after 21 days of cold storage pointed to more significant phenomena in the control, while the pre-treatments with hot H_2O, O_3 and UV-C were effective in slowing down these effects. These findings are opposite to the research, which showed that UV-C and O_3 suppressed the decrease of firmness in cherry tomato (Bu et al., 2013). Chen and Zhu (2011) confirmed that aqueous ClO_2 plus ultrasound inactivated the cell wall enzymes and inhibited the softening. The enzyme activity levels of polygalacturonase and pectin methylesterase were reduced, and the relative expression levels of related genes were suppressed by the UV-C pre-treatment, which controlled the components of the cell wall, including protopectin, acid-soluble pectin and cellulose (Bu et al., 2013). These results may indicate different maturity performances caused by different types of fruits and vegetables.

The hue angle value, ranging from green (180°) to yellow (90°), indicates a decrease in the intensity of greenness and an increase in yellowness. Slight drops in hue angle value were detected both in the top and bottom portions of all pre-treatments after 21 days of cold storage (Table 3). Mainly, in the ClO_2, O_3 and UV-C pre-treatments lower hue angle values were obtained both in the tips and stems. The same effect was observed in coated asparagus with the silver nanoparticles-PVP (polyvinylpyrrolidone) during post-harvest storage (An et al., 2008). Likewise, the total colour difference (ΔE^*) of all sterilisation pre-treatments was calculated and showed a statistically significant difference in tips but not in stems. The total deviation from initial samples was lower than the control, especially in the NaClO (11.86 of tip and 9.53 of stem) and hot H_2O (13.43 of tip and 8.60 of stem) pre-treatments. It is consistent with the change of hue angle values. The reduction of the hue angle value probably correlated with the decrease in chlorophyll content over the cold storage period. Notably, the hot H_2O pre-treatment was demonstrated to have a significant retarding effect on decreases in hue angle value. The hue angle of stems was lower than that of tips in all groups over the cold storage period.

The EL reflects the integrity and permeability of the cell membrane and, to some extent, represents the damage of fruits and vegetables. The EL was measured immediately after sterilisation pre-treatments and on the last storage (the 21st) day. Significant differences between the control group and sterilisation groups were observed (Table 3). Asparagus pre-treated with different sterilisation methods showed significantly higher EL values than those of the untreated with 0.14–0.39 higher than those of the control immediately after sterilisation pre-treatments but lower after 21 days of cold storage. These findings indicated that a different degree of damage caused by sterilisation occurred after sterilisation pre-treatment, but the sterilization pre-treatment was able to maintain the asparagus quality during the subsequent storage. The highest EL was with the O_3 pre-treatment with an EL value of 0.83, i.e. 0.39 higher than the control, possibly due to cell wall and protopectin breakdown, which results in releasing the tissue fluid and increases the ionic mobility (Sarang et al., 2008). Moreover, among the sterilisation pre-treatments, the ClO_2 and hot H_2O pre-treatments caused less damage with lower EL value. No significant differences were found between any of the sterilisation pre-treatments.

Correlations analysis. The correlations of visual quality (VQ), off-odour, fresh weight (FW) loss rate, colour, firmness, soluble solids content (SSC), electrolyte leakage (EL) and microorganisms were analysed on the final day of cold storage (Table 4). After 21 days of cold storage, significant correlations ($P \leq 0.01$) were observed between VQ and the growth of total aerobic bacteria as well as between off-odour and firmness of asparagus spear tips. The surface feature of appearance, off-odour, colour change, decay, water loss, cracking and other phenomena were taken into account when the VQ was scored.

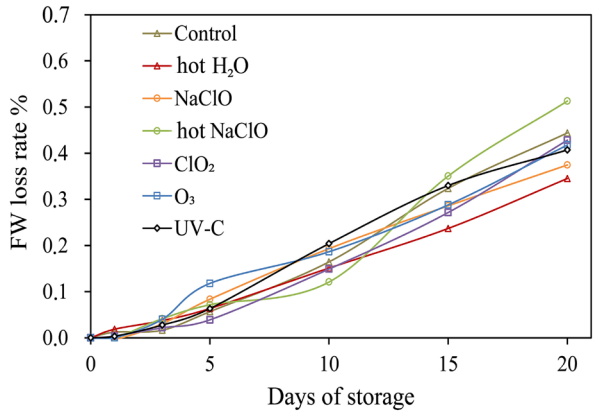
Table 4. Correlation analysis between sensory quality (visual quality and off-odour), physiological and biochemical traits and the development of microorganisms of green asparagus during cold storage

	VQ	Off-odour	FW	C-T	C-S	F-T	F-S	SSC	EL	*E. coli*	TAB	Y and M	CO_2	O_2	C_2H_4
VQ	1.00	–0.47	–0.04	0.54	0.57	–0.46	–0.06	–0.03	–0.26	0.17	0.918**	–0.31	0.16	–0.21	–0.32
Off-odour			0.28	–0.35	–0.66	0.937**	0.08	0.34	0.05	0.11	–0.22	–0.30	0.63	0.14	0.24
FW				0.02	–0.18	0.04	–0.42	–0.71	0.43	0.45	0.04	0.48	–0.08	–0.22	0.23
C-T					0.784*	–0.47	–0.26	–0.14	–0.70	–0.42	0.69	–0.28	0.26	–0.05	–0.52
C-S						–0.75	–0.34	–0.07	–0.34	–0.37	0.52	–0.21	–0.20	0.21	–0.13
F-T							0.40	0.54	0.05	0.00	–0.29	–0.28	0.59	–0.03	0.09
F-S								0.56	0.01	–0.46	–0.20	0.11	0.04	–0.66	–0.53
SSC									–0.25	–0.44	–0.02	–0.66	0.42	0.19	–0.05
EL										0.41	–0.51	0.60	–0.65	–0.08	0.64
E. coli											0.16	0.06	0.01	0.15	0.49
TAB												–0.50	0.48	–0.10	–0.36
Y and M													–0.755*	–0.54	–0.03
CO_2														0.10	–0.28
O_2															0.70
C_2H_4															1.00

Note. VQ – visual quality, FW – fresh weight loss rate, C-T – colour (hue angle value) of asparagus tips, C-S – colour (hue angle value) of asparagus stems, F-T – firmness of asparagus tips, F-S – firmness of asparagus stems, SSC – soluble solids content, EL – electrolyte leakage, *E. coli* – *Escherichia coli*, TAB – total aerobic bacteria, Y and M – yeast and mould; * – means significant correlation at 0.05 level ($p \leq 0.05$), ** – means extremely significant correlation at 0.01 level ($p \leq 0.01$).

These results indicated that the development of total aerobic bacteria was one of the important factors aggravating the deterioration of VQ. Additionally, the concentration of CO_2 in MA package was negatively correlated with the development of yeast and moulds with a correlation coefficient of −0.755 ($P \leq 0.05$). This was likely related to the sterilisation effect of CO_2 (Rao et al., 2016). Above all, the quality properties of green asparagus were significantly correlated. Sterilisation pre-treatments influence the physiological and biochemical traits of green asparagus. All these factors together determined the shelf-life of green asparagus.

Sensory quality. The FW loss rate was measured over the cold storage period until the 21st day. The results are presented in Figure 2. The FW loss rates for each pre-treatment and control steadily increased during the cold storage period.

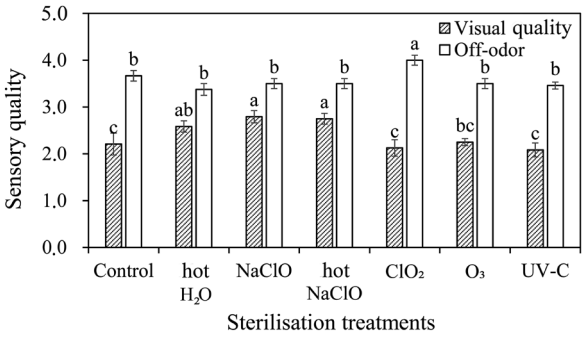


Note. Vertical bars represent the mean ± SE (n = 5).

Figure 2. Effects of sterilisation pre-treatments on the fresh weight (FW) loss rate of green asparagus during cold storage

Depending on the data after 21 days of cold storage, significant differences between sterilisation pre-treatments and the control were found. The lowest FW loss rate was noted from the hot H_2O pre-treatment, and the highest FW loss rate was noted from the hot NaClO pre-treatment with values of 0.35 and 0.51, respectively. In addition, the hot H_2O pre-treatment suppressed the increase in the FW loss rate in comparison with the control, which is consistent with the report in mango (Angasu et al., 2014). Most research has stated that the FW loss rate of green asparagus is mainly related to the high transpiration rate and the difference in vapour pressure between the surface and the atmosphere (Techavuthiporn, Boonyaritthongchai, 2016). The reason for the lower FW loss in green asparagus may be because the hot H_2O pre-treatment led to minimal damage, and the lower FW loss was also showed in strawberry treated with 45°C hot water adding salicylic acid, compared to 25°C water (Shafiee et al., 2010). After 21 days of cold storage, no dehydration signs in green asparagus were observed, because the FW loss rate was much lower than the critical levels in all groups.

A slight to moderate level of off-odour was detected in all groups after 21 days of cold storage. After 21 days, none of the groups was marketable showing scores higher than 3.0, which indicates the borderline of acceptability (Fig. 3). Among all groups, the worst off-odour was observed in the ClO_2 pre-treatment with a score of 4.0; no significant differences were found among the other groups. Fishy and rotten-egg off-odour in baby spinach is mainly related to the production of chemicals such as alcohols, sulphur compounds, methanethiol, dimethyl trisulfide, β-ionone and acetaldehyde (Díaz-Mula et al., 2017).



Note. Vertical bars represent the mean ± SE (n = 5); means (n = 5) followed by the same letter in the column are not significantly different ($P > 0.05$) according to Duncan's multiple range test; visual quality was scored on a 5 to 1 scale, where 5 – best and 1 – worst; off-odour was scored on a 1 to 5 scale, where 1 – no off-odour and 5 – extremely strong off-odour.

Figure 3. The sensory quality of green asparagus after sterilisation pre-treatments and after 21 days of cold storage

When the overall VQ was evaluated, it was observed that during cold storage the VQ score decreased to the threshold of marketability (<3.0). Significant differences in VQ were observed among the different sterilisation pre-treatments (Fig. 3). The VQ levels in the ClO_2 and UV-C pre-treatments after 21 days of cold storage were worse than those of the control because of the colour changes and the shrinking of stems, while others were higher than the control. The effectiveness of the surface disinfectants NaClO (hot and normal temperature solution) and hot water in preserving VQ was indicated by the higher scores assessed by a panel. In general, the sterilant of NaClO both hot and normal was the best with higher sterilisation efficiency and better sensory quality.

Conclusions

1. The results of the experiment revealed that the application of different surface disinfectants and sterilisation pre-treatments was efficient in prolonging the shelf-life of green asparagus up to 21 days.

2. All sterilisation pre-treatments inhibited the growth of microorganisms with a lower number on the initial day after sterilisation, compared to the control: total aerobic bacteria – 4.10 log CFU g^{-1}, yeast and mould – 1.43 log CFU g^{-1}. According to the experimental data, different effects were noted due to the different kinds of microorganisms and disinfectants.

3. After 21 days of cold storage, the ozone (O_3), chlorine dioxide (ClO_2) and sodium hypochlorite (NaClO) pre-treatments were remarkable at inhibiting microorganisms with the lowest number of total aerobic bacteria (5.23 log CFU g^{-1}), yeast and mould (0.67 log CFU g^{-1}) and *Escherichia coli* (3.67 log CFU g^{-1}), respectively. Considering the growth of all microorganisms, the NaClO pre-treatment provided the most remarkable sterilisation effect (10.24 log CFU g^{-1}). The hot H_2O pre-treatment showed a better effect in maintaining the quality of green asparagus with the highest soluble solids content (SSC) (4.45), hue angle value in tips (121.21°) and in stems

(118.76°), lower electrolyte leakage (EL) (0.16) and fresh weight (FW) loss rate (0.35%). The higher visual quality (VQ) scores were obtained in the NaClO (2.79), hot NaClO (2.75) and hot H_2O (2.58) pre-treatments and no significant difference ($P \leq 0.05$) among three of them, but showed statistically significant difference with other pre-treatments. The hot H_2O pre-treatment led to the lowest off-odour (3.38), while the worst odour was in the ClO_2 pre-treatment (4.0). These sterilisation pre-treatments described in this study probably affected the SSC and hue angle significantly with significant correlations ($P \leq 0.01$) and the statistically significant difference between different pre-treatments.

Acknowledgements

This study was supported by the IPET through Export Promotion Technology Development Program, funding from the Ministry of Agriculture, Food and Rural Affairs (Nos 114092-03 and 117035-03). Authors L.-X. Wang and I.-L. Choi contributed equally to this work.

Received 30 03 2020
Accepted 18 09 2020

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Paviršiaus sterilizavimas taikant cheminius ir fizinius metodus turi įtakos mikroorganizmų kiekiui ir smidrų kokybei

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Santrauka

Tyrimo metu buvo vertintas vaistinio smidro (*Asparagus officinalis* L.) paviršiaus dezinfekavimo priemonių ir sterilizavimo metodų poveikis antimikrobiniam efektyvumui ir daržovių kokybės išsaugojimui jas sandėliuojant šaltyje iškart po išankstinio sterilizavimo ir po 21 parą laikymo šaltai. Paviršiui sterilizuoti naudotas karštas vanduo, natrio hipochlorito (NaClO) tirpalas, karštas NaClO tirpalas, ozonas (O_3), chloro dioksidas (ClO_2) ir veikimas UV-C; kontrolinės grupės smidrai nebuvo iš anksto apdoroti. Įvertinta smidrų vizualinė kokybė, fiziologinės bei biocheminės savybės ir koreliacija tarp išankstinių sterilizavimo procedūrų bei smidrų kokybės. Tyrimo metu visi dezinfekantai ir išankstinio apdorojimo metodai mikroorganizmų kiekį sumažino veiksmingiau nei kontrolinės grupės smidrų. Išankstinis apdorojimas O_3, ClO_2 ir NaClO buvo itin efektyvus slopinant bakterijų (ypač *Escherichia coli*), mielių ir pelėsių, turinčių mažesnį kiekį mikroorganizmų, augimą 21 parą laikant šaltai. Plaunant karštu vandeniu buvo geresnė smidrų maistinė bei vizualinė kokybė ir padaryta minimali žala smidrams, kurie išsiskyrė didesniu tirpių kietųjų medžiagų kiekiu bei spalvos tono verte ir mažesniu elektrolitų nutekėjimu. Nė vienoje iš pakuočių nebuvo nustatyta reikšminga įtaka smidrų kietumui ir pakuotės atmosferos sudėčiai. Reikšminga koreliacija buvo nustatyta tarp išankstinių sterilizavimo būdų ir tirpių kietųjų medžiagų kiekio bei spalvos.
Visi išankstinio apdorojimo būdai veiksmingai slopino mikroorganizmų vystymąsi ir žymiai pailgino smidrų galiojimo laiką, tačiau efektyvesnis buvo išankstinis apdorojimas O_3, ClO_2 ir NaClO.

Reikšminiai žodžiai: chloro dioksido dujos, karštas vanduo, ozono dujos, natrio hipochlorito tirpalas, UV-C.